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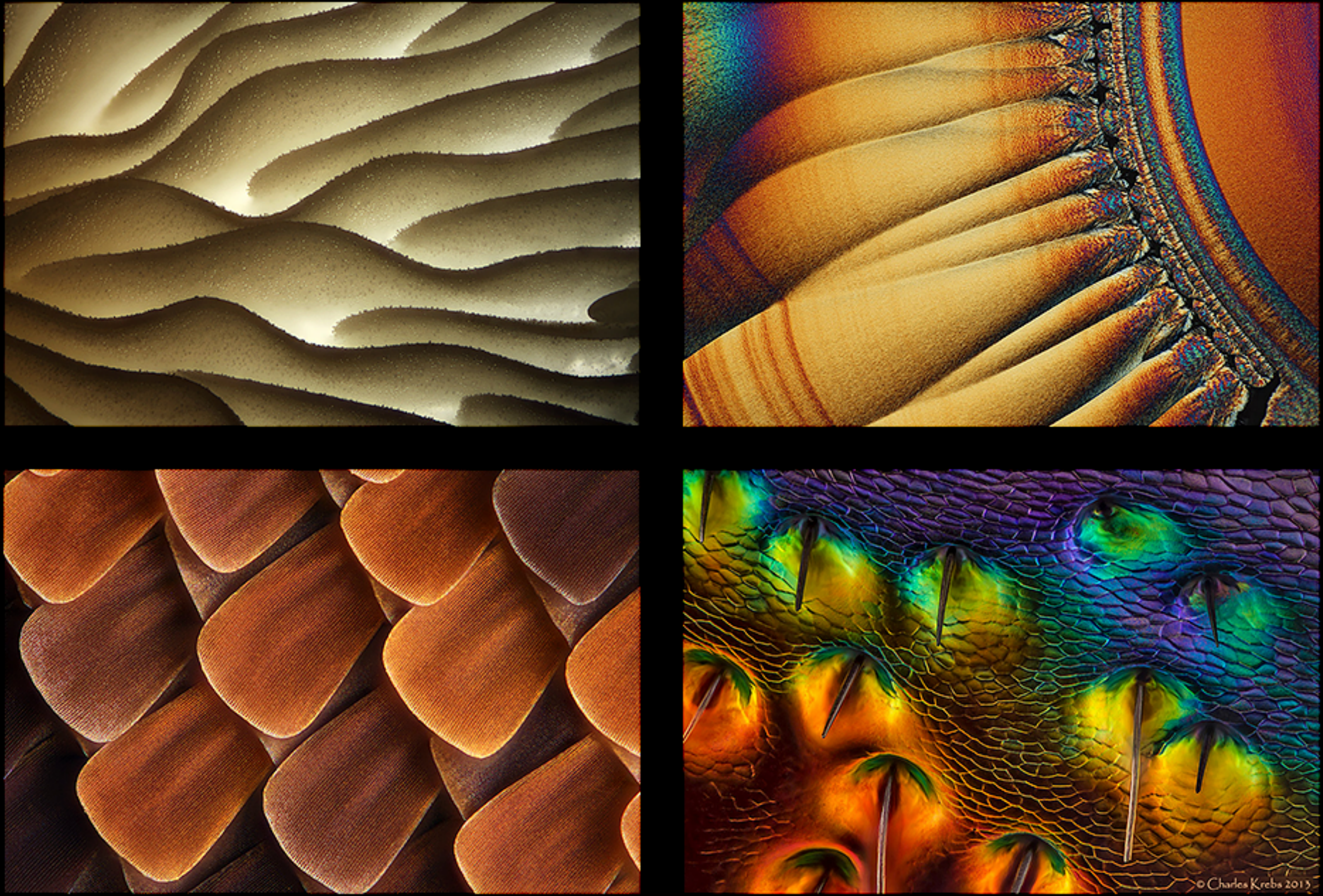
Charles Krebs

Beyond Macro Photography: Into the Microscopic World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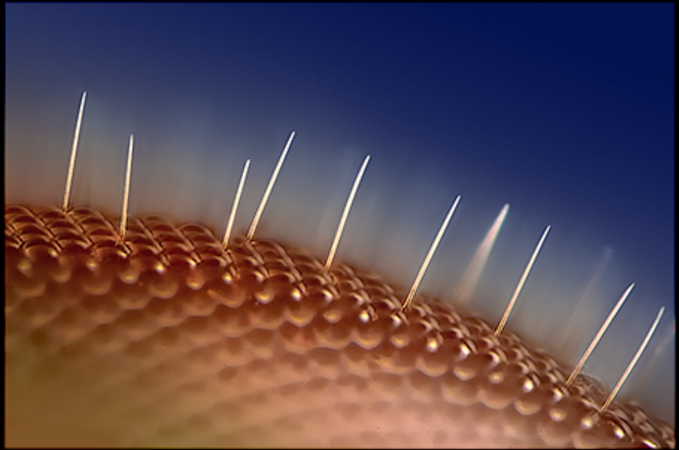

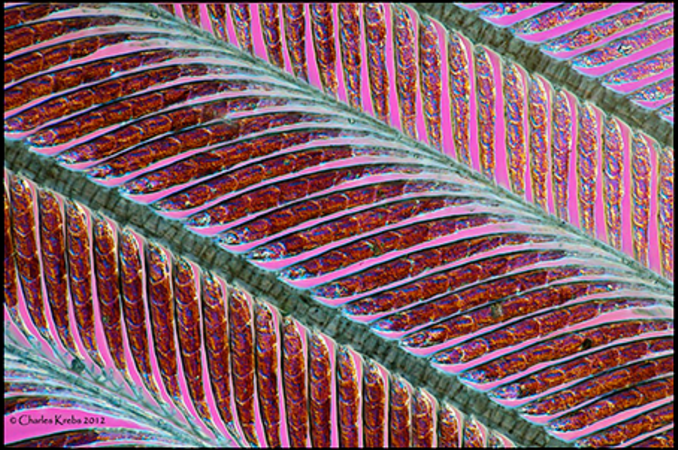
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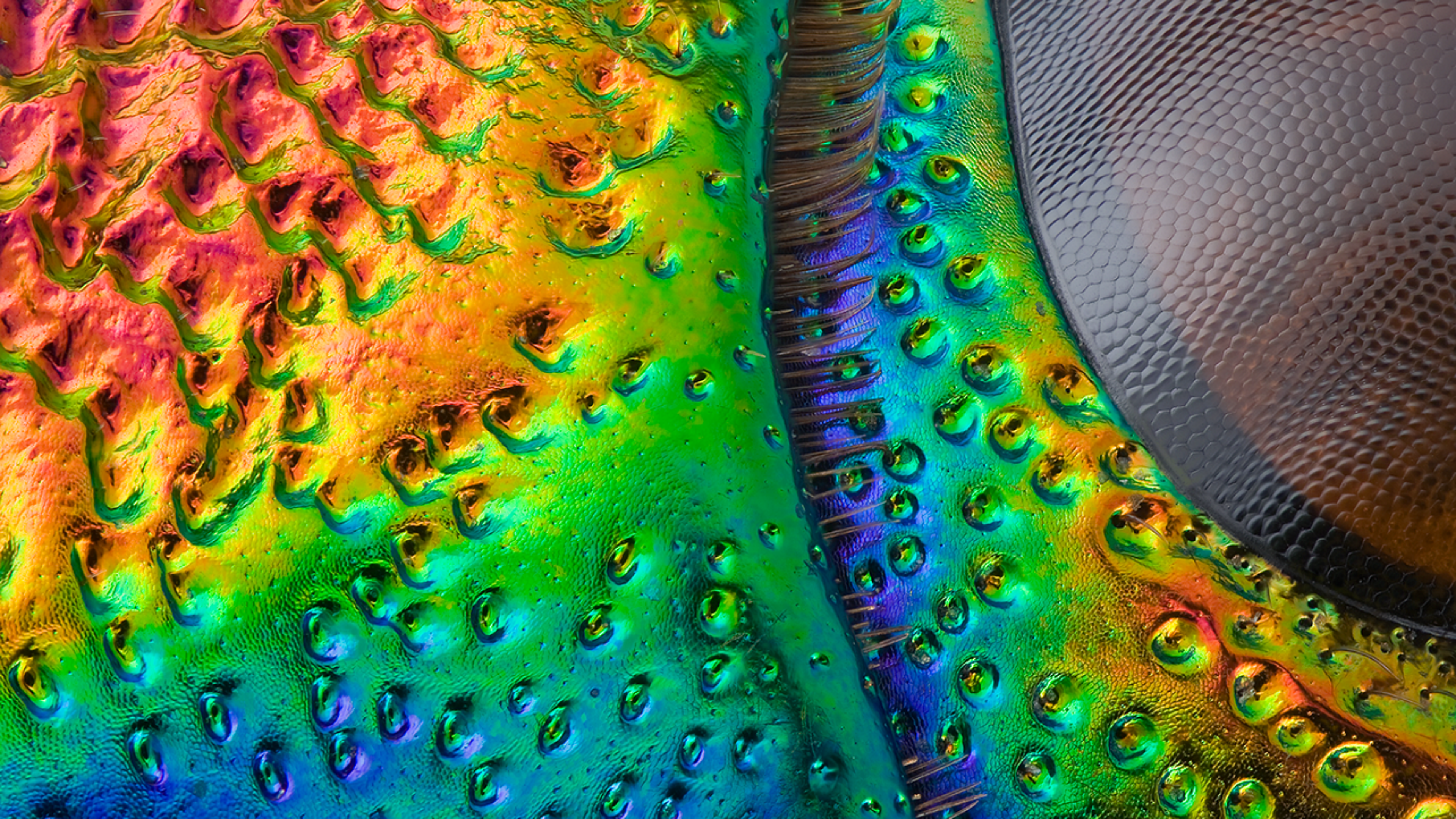


Transition from Macro to Microphotography

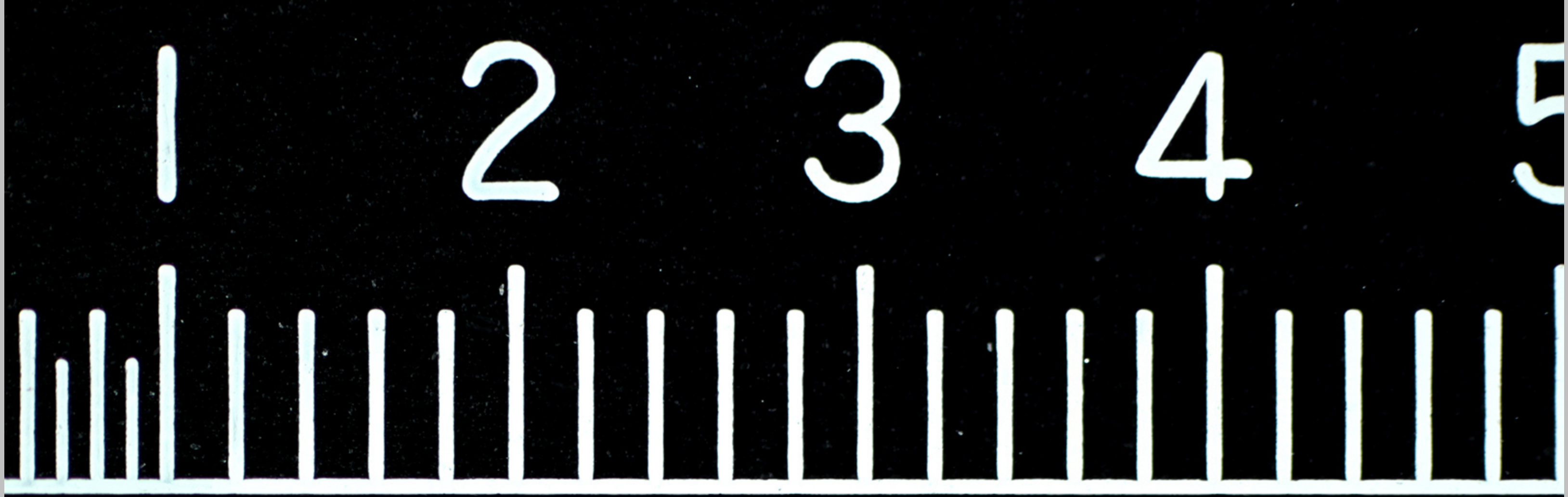
Beyond Macro Photography

- Macro lenses can be focused to .5X or 1X
- A variety of techniques can be used between 1X and 5X
- For magnifications of about 5X and higher, the best results are obtained utilizing microscope objectives

Into the Microscopic World

- Difficulties encountered in close-up photography become extreme
- Optics, and some necessary equipment not familiar to most photographers
- Focus stacking becomes necessary in many cases
- The precision of a true microscope greatly facilitates working at very high magnifications


7



magnification = image size / object size
magnification = recorded width / sensor width

magnification = 4.46 / 22.3

magnification = 5X

Between 1X and 5X

- Supplementary close-up lenses
- Stacked lenses
- Reverse mounted lenses
- Canon 65mm f/2.8 MP-E, 1X-5X lens


RAYNOX MACROSCOPIC LENS Model M-150
MADE IN JAPAN ϕ49

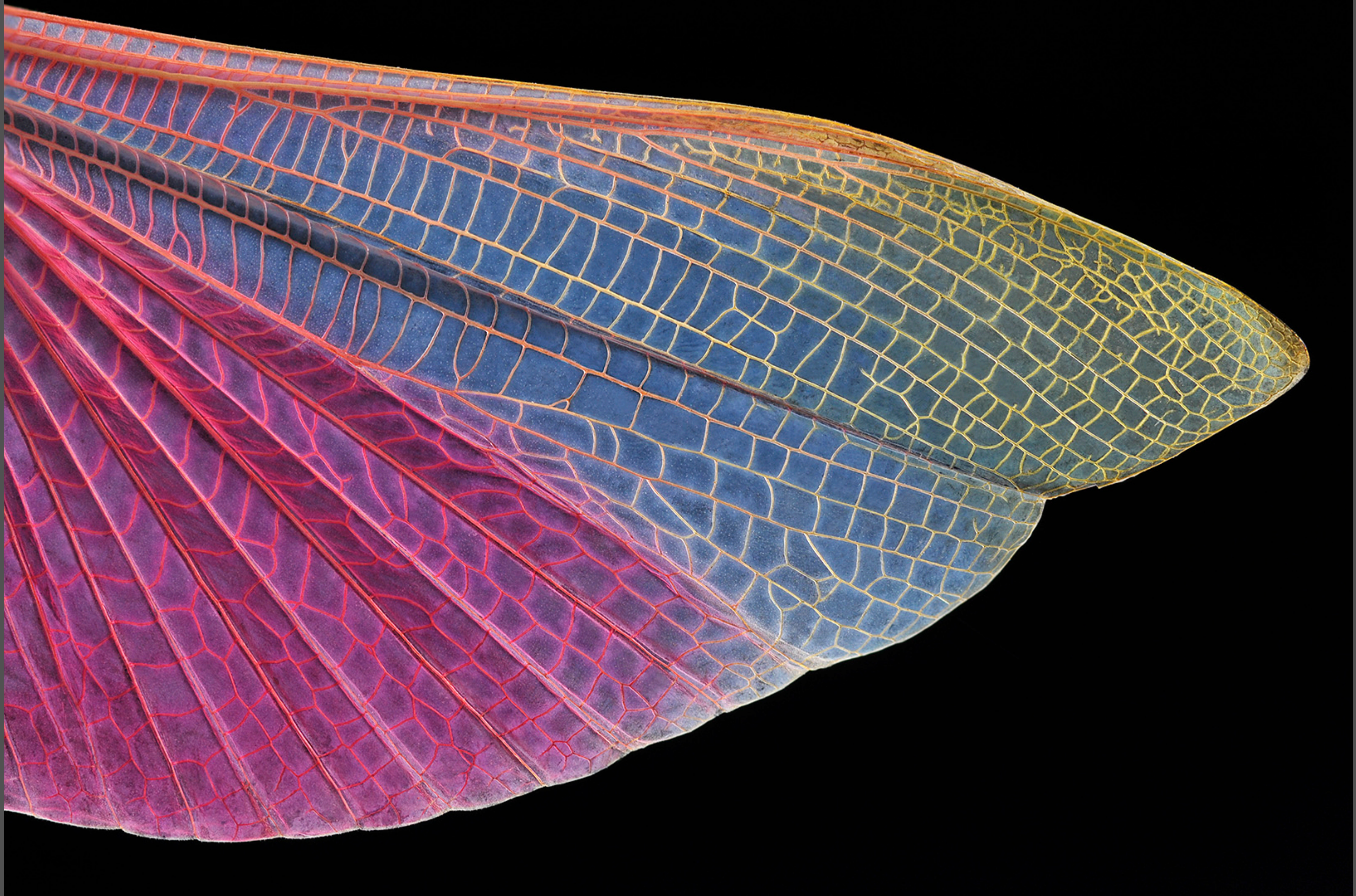

Nikon
D300
RAYNOX MACROSCOPIC LENS Model M-150
MADE IN JAPAN
LIMIT -
FULL -


Nikon
D300
QUAL
WB
ISO
Nikon
AF NIKKOR
50mm
1:1.8
A M
VR
ON OFF
VIDEO OUT
DC IN


Canon
EOS 50D
Nikon
AF NIKKOR
50mm 1:1.8
for Canon AF Japan
VIDEO OUT


4/80
Schneider
4 ·5.6· 8


LENS MP-E 65mm 1:2.8 1-5x
MP-E 65mm
EOS 50D
VIDEO OUT
HDMI
Really Right Stuff


IN GOD
TRUST
LIBERTY
2011
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2011
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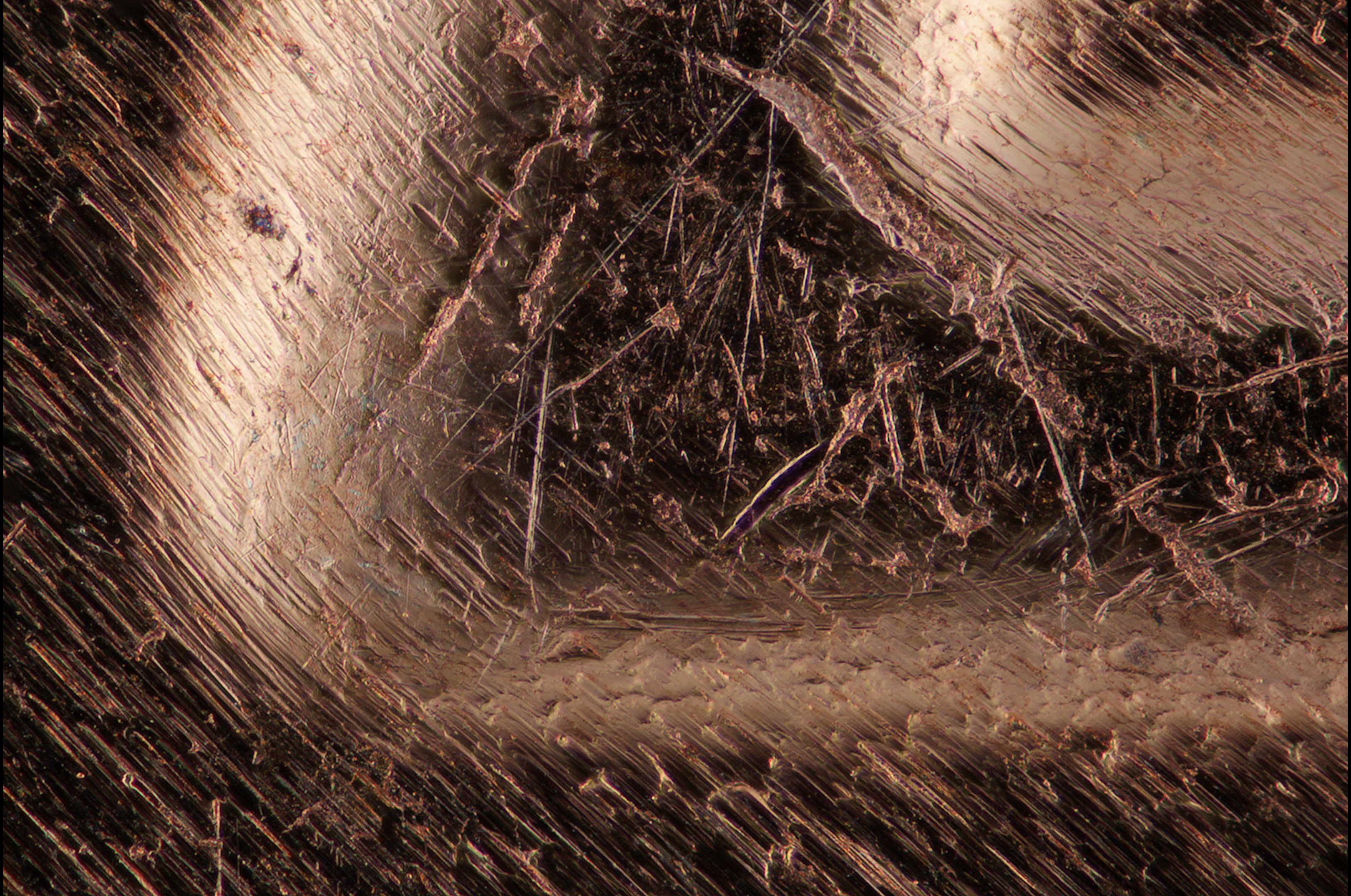
20

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The Three Main Optical Issues

- Depth-of-field
- Diffraction
- Vibration

Depth–of-field

•As magnification increases, depth-of-field decreases very dramatically

•Smaller apertures can increase DOF, but increases diffraction and can significantly lower resolution

•Careful subject orientation will make best use of available DOF

•Combining the sharpest portions of images taken at various focus depths, or “focus stacking”, is a very effective way to create images of 3-dimensional subjects at high magnifications

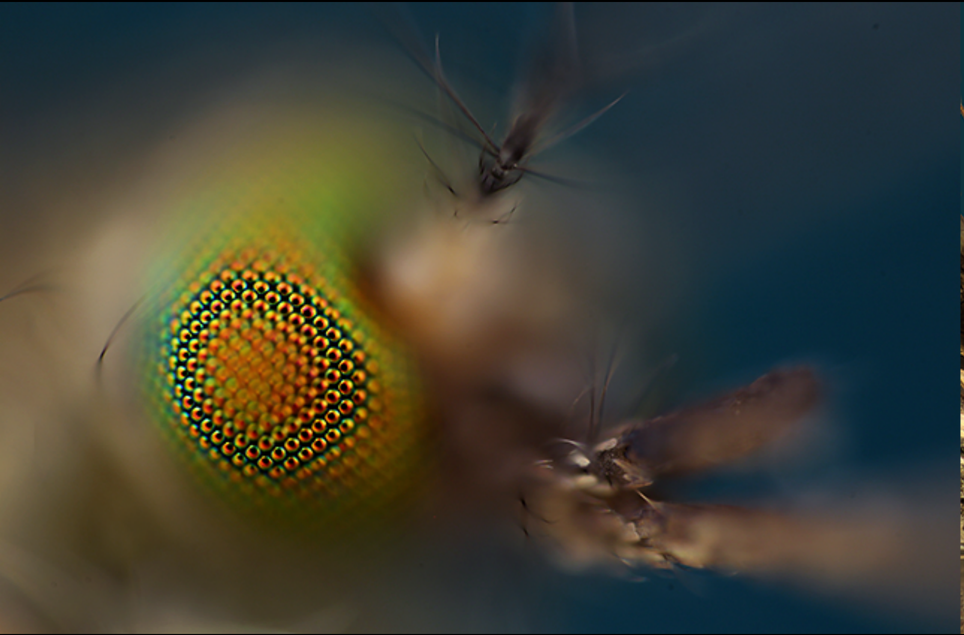
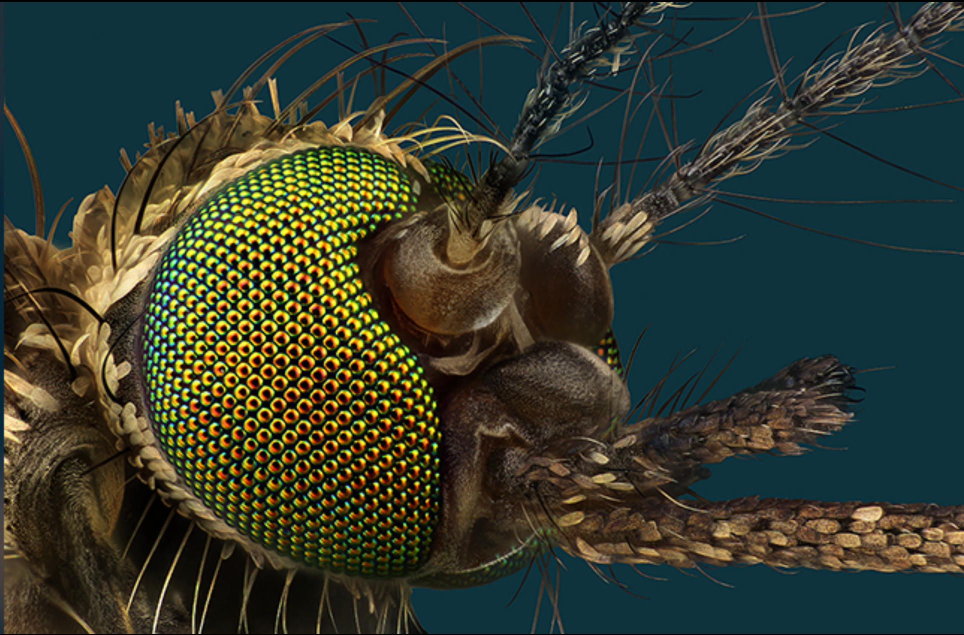
Single image

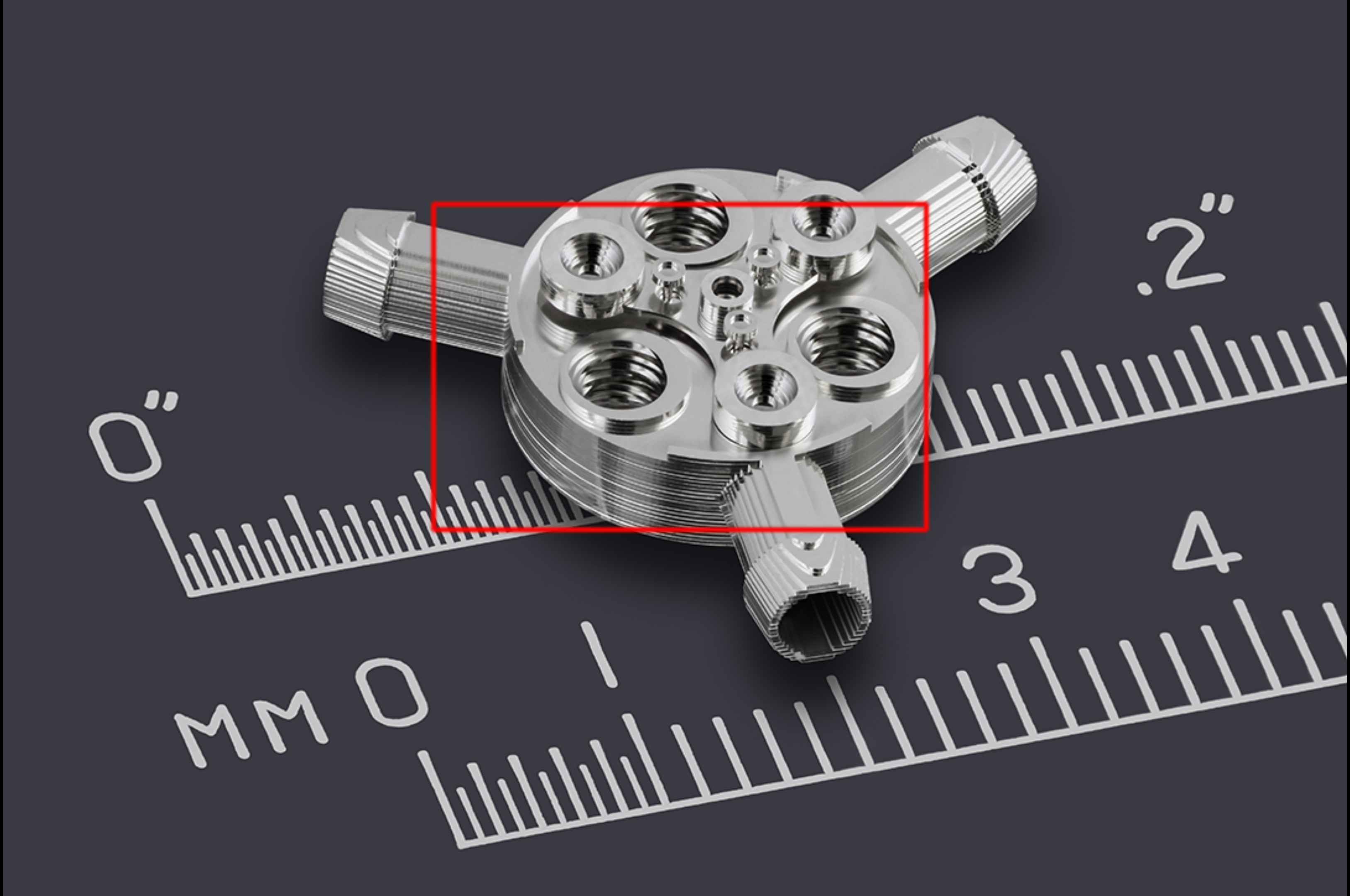
86 image stack

Moth Eggs

Single image

291 image stack


0"
.2"
MM 0
1
3
4

Single image

332 image stack

Mag (objective)	micron	mm	inch
1X (macro lens)	1000	1	0.039370
5X (5/0.15)	30	0.03	0.001181
5X (lens at f/2.8)	30	0.03	0.001181
10X (10/0.28)	8	0.008	0.000315
20X (20/0.40)	3.4	0.0034	0.000134
50X (50/0.50)	2.2	0.0022	0.000087
50X (50/0.80)	0.86	0.00086	0.000034

A standard CD is 1.2mm (1200 microns) thick.
A pin-head has a diameter of about 1.0 mm (1000 microns).
A typical grain of salt is approximately 0.3 mm (300 micron) on a side.
A red blood cell is 6-8 micron in diameter
A sheet of 20lb copy paper is 97-114 microns thick.

Optical issues

- Depth-of-field
- Diffraction
- Vibration

Diffraction

•Diffraction is caused by the wave nature of light, and in photography is directly related to effective aperture size

•As diffraction effects increase, the resolution and contrast of a photographic image is reduced

•Common f/#'s that we are familiar with are only valid when the lens is focused at "infinity" (∞)

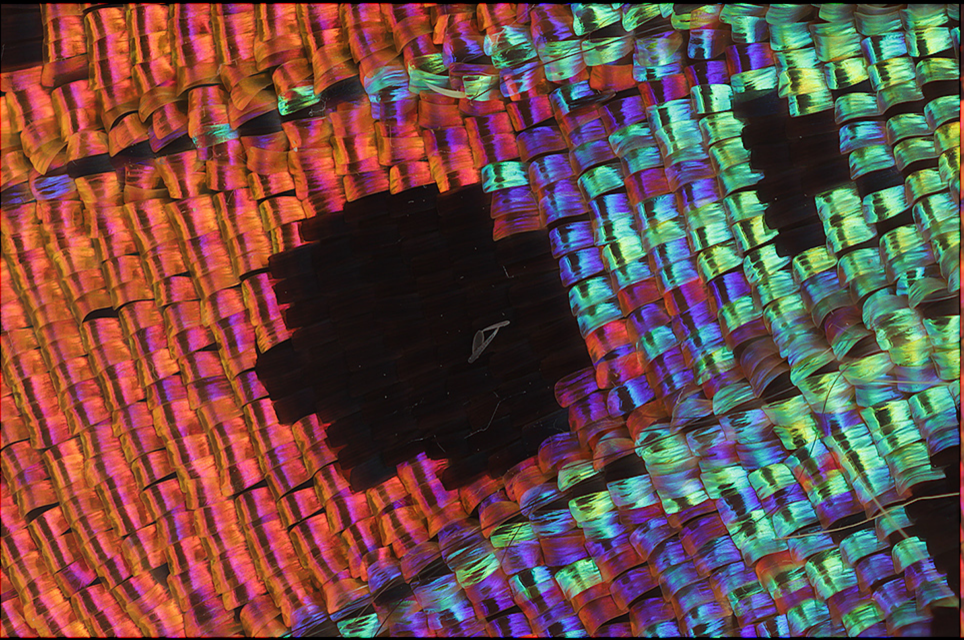
•The actual working, or *effective aperture*, becomes smaller when a lens is focused closer by extension

Effective aperture (f_{eff})

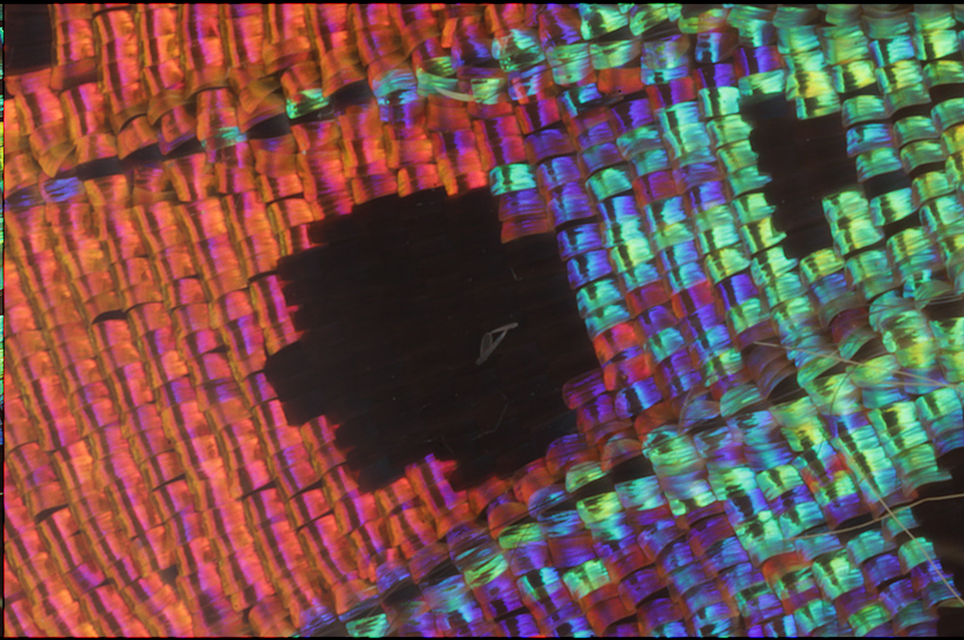
$$f_{eff} = f\,(m+1)$$

where f = f-number marked on lens, m = magnification on sensor (Note: This is a basic equation for symmetrical lenses, and is not valid for all lenses)

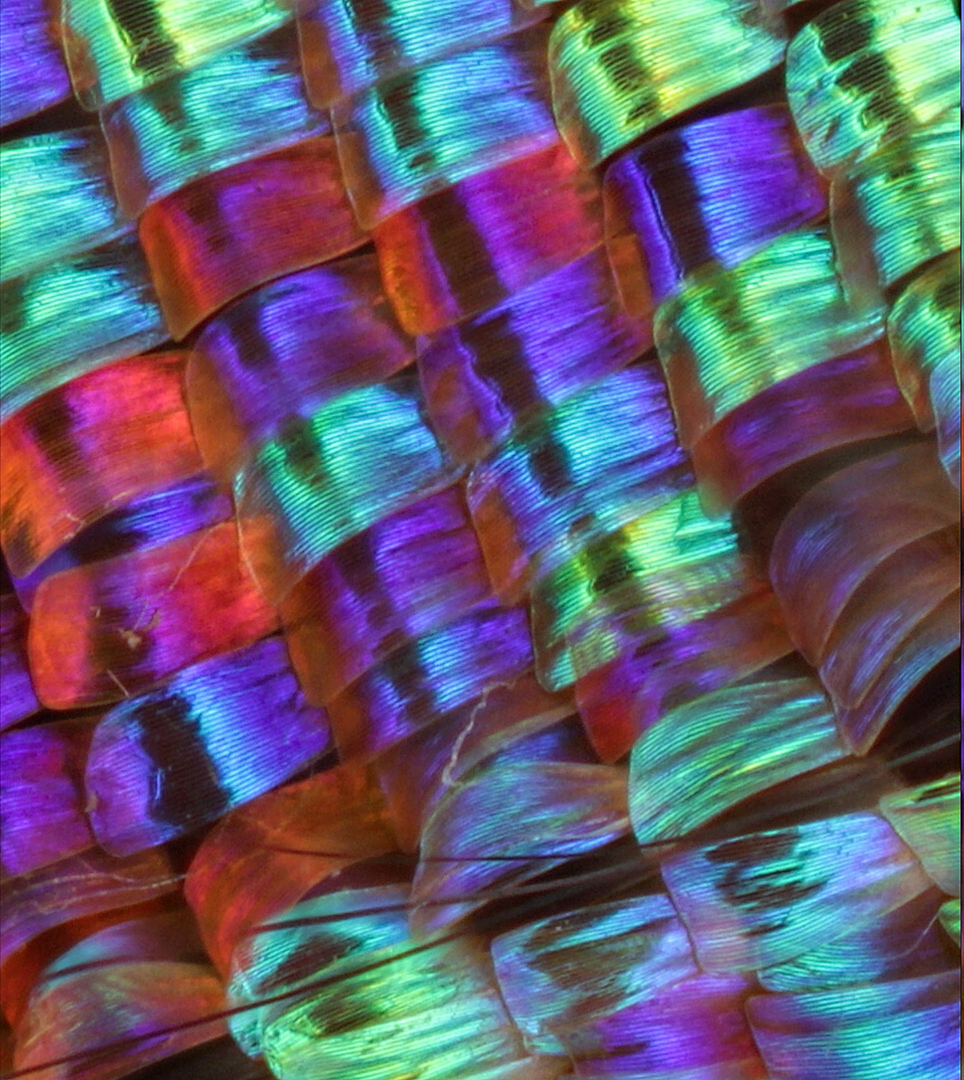
5X on APS-C sensor

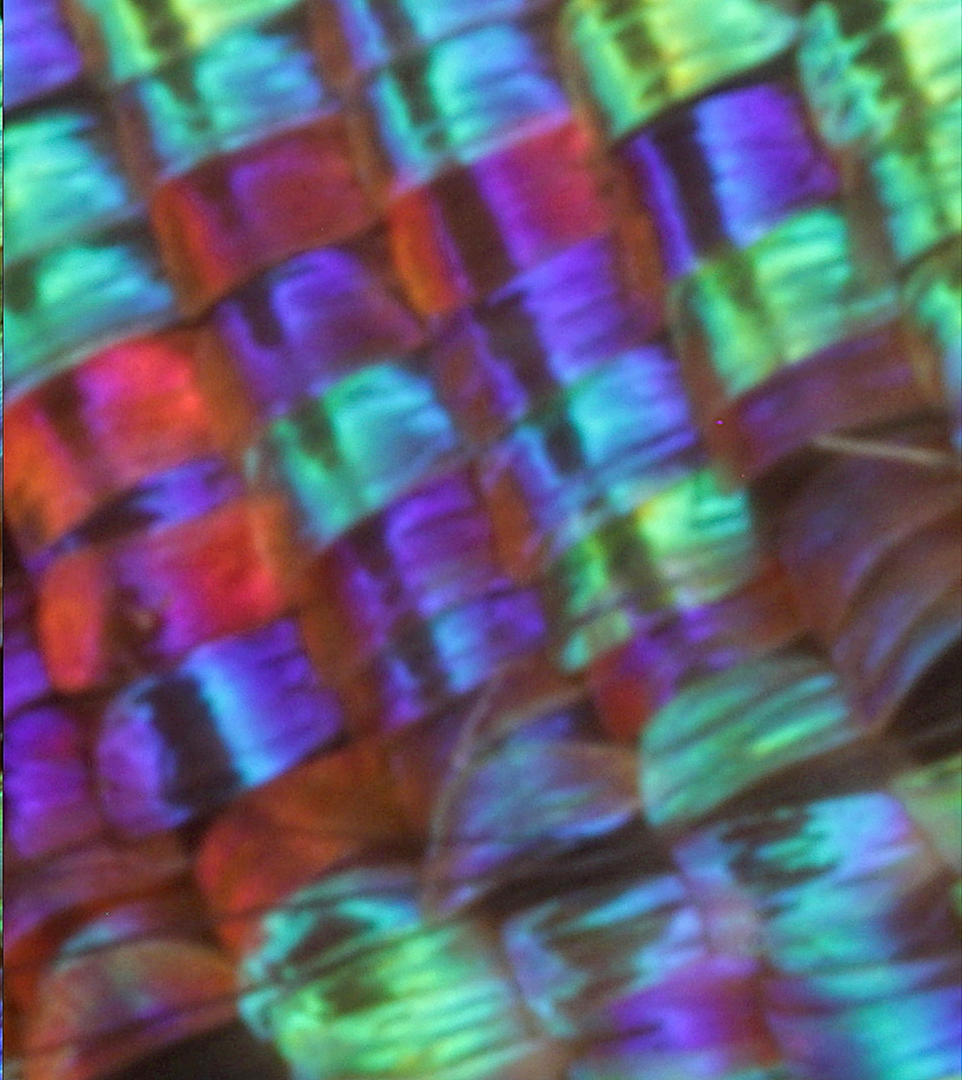


17 image stack
lens at f/3.5
effective aperture f/21



Single image
lens at f/16
effective aperture f/96





Effective aperture benchmark for sensor size

- Micro 4/3: $f_{eff}/16\text{-}22$
- APS-C: $f_{eff}/22$
- 35mm: $f_{eff}/32$

At these benchmarks diffraction has already had some effect on image quality, but they reflect a reasonable compromise given available optical solutions, and average image usage. *Your image uses may warrant a larger or smaller value.*

Optical Issues

- Depth-of-field
- Diffraction
- Vibration

Vibration

- SLR mirror mechanism
- Mechanical shutter
- Handling. After a camera control has been touched, or the focus moved to a new point, equipment vibrations must be given some time to settle out
- External, or environmental, sources must be assessed

Camera shutter vibration

- A fully mechanical shutter will negatively impact image quality
    - Use long shutter speeds from 1 to 4 seconds
    - Use electronic flash
        - Use long exposure with second curtain sync electronic flash

- Some cameras now have electronic first shutter curtain (EFSC)

- A few cameras now have fully electronic shutter option

Choosing Microscope Optics

- Most conventional lenses used for macro have a maximum f-number of about f/2.8, but typically need to be closed down about 1-1.5 stops for the best quality

- As a result, if we want an effective aperture no smaller than $f_{eff}/22$, our useful magnification with that optic is in the range of 3X to 5X

- Microscope objectives can have large light gathering ability that provides a large effective aperture, so resolution is higher than with conventional macro optics

Microscope objectives

- Light gathering ability denoted by **Numerical Aperture** (NA), not f-number
- NA… key to resolution, analogous to maximum aperture
- Fixed magnifications such as 4/5X, 10X, 20X, 40/50X, 60X, 100X
- Designed to be used on a microscope, some can be used on bellows/tubes/lenses

10/0.28 microscope objective

Roughly equivalent to a 20mm f/1.6 lens
At 10X, Effective Aperture = f/18

Regular lens at 10X

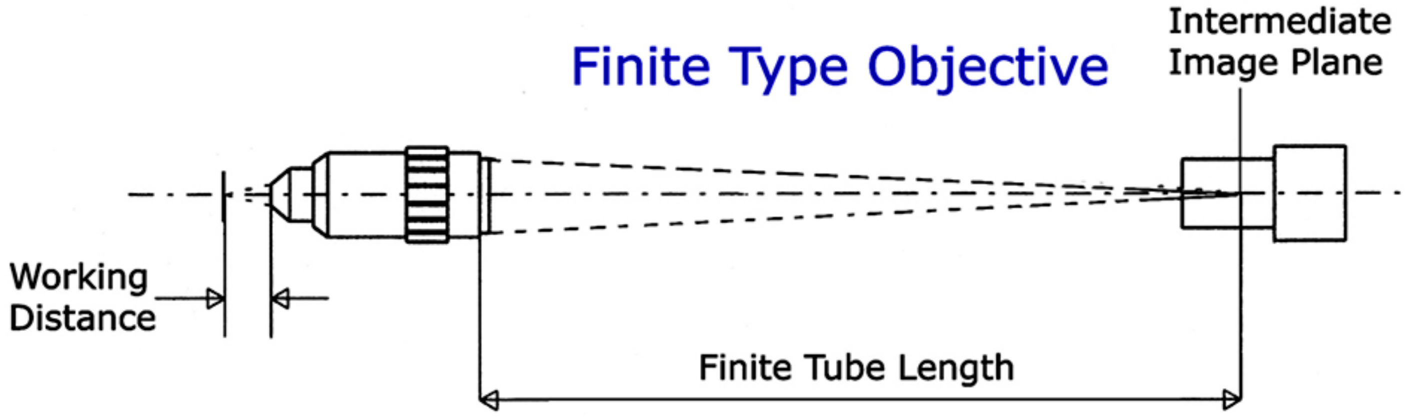
Marked f/2.8, actual Effective Aperture = f/31
Marked f/4, actual Effective Aperture = f/44

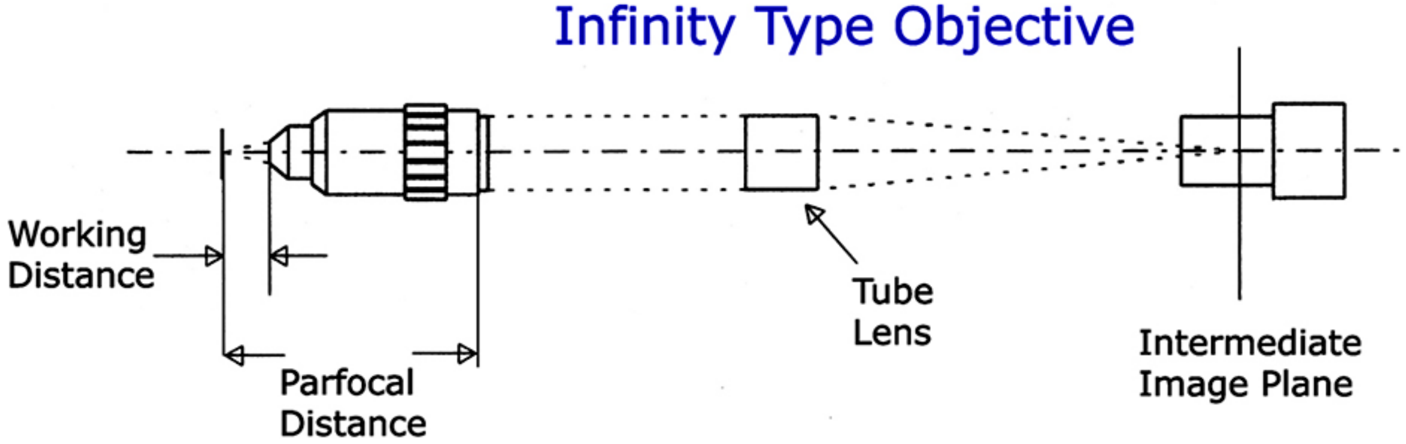
20/0.40 microscope objective

Roughly equivalent to a 10mm f/1.2 lens
At 20X, effective Aperture = f/24

Regular lens at 20X

Marked f/2.8, actual Effective Aperture = f/59
Marked f/4, actual Effective Aperture = f/84


Finite Type Objective
Intermediate
Image Plane
Working
Distance
Finite Tube Length


Infinity Type Objective
Working
Distance
Parfocal
Distance
Tube
Lens
Intermediate
Image Plane

Important objective characteristics

Degree of correction

•Different designed levels of optical and color correction… plan and non-plan, achromat, semi-apochromat, and apochromat

Biological and Industrial objectives

•Biological are historically intended for transmitted lighting methods, and typically with a coverglass covered subject

•Industrial (or metallurgical) are primarily intended for opaque subjects that are not covered by a coverglass

Working Distance (WD)

- The distance between the tip of the objective and the subject

- Highly variable and often far too small to be used off of a microscope with subjects using reflected light.

- WD typically not a major concern on a biological microscope

Limited image circle

- Microscope objectives produce a relatively small image circle

- 18-20mm diameter image circle for older ones, 25-27mm diameter for newer super wide-field designs

- Pay attention to sensor size/image circle size. In many cases the actual usable image is significantly larger than specified

Need for corrective eyepieces on some finite objectives

- Many older objectives used chromatically corrective eyepieces and photoeyepieces as the last stage of color correction. Avoid these for use with bellows/tubes

- No current infinity corrected optical systems use chromatically corrective eyepieces

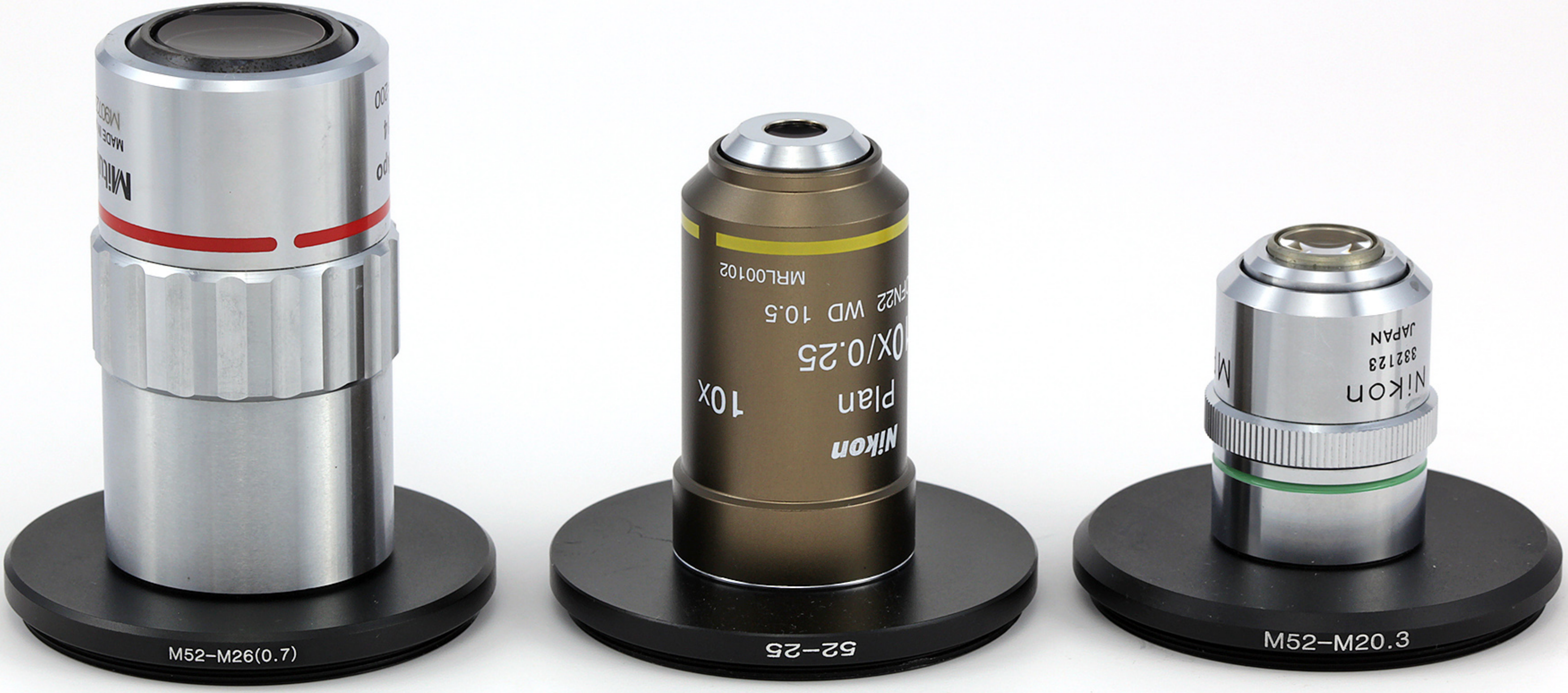
- Two brands, Leica and Zeiss, currently utilize color corrective tube lenses. Best to avoid these mounted on a camera lens or bellows/tubes

M Plan Apo
5× / 0.14
∞ / 0 f=200
Nikon
Plan
10x/0.25
∞/- WD 10.5
Plan 10 / 0.30
160/0.17
OLYMPUS
LMPlanFL N
20x /0.40
∞/0/FN26.5
MPlan 20
0.4 ELWD
210/0


OLYMPUS
LMPlanFL N
20x /0.40
∞ / 0 /FN26.5
MPlan 20
0.4 ELWD
210 / 0


Nikon
Plan
10x/0.25
∞/- WD 10.5
MADE
Plan 10 /
160/0.17

Microscope Optics Setups



Mitutoyo
M26 x 0.7

Nikon CFI60
M25 x 0.75

RMS thread
M20.32


Nikon
D300
Mitutoyo
MADE IN JAPAN
A
M
VR
ON
OFF


Mitutoyo
MADE IN JAPAN
M90727907


Canon
46mm → 52mm


Canon
EOS 50D
3
2
for Canon AF Japan
plan 10/ 0.30
160/0.17
VIDEO OUT


Canon
EOS
50D
plan 10 / 0.30
160/0.17


Plan
10 /
160/0.17

Mitutoyo M Plan Apo, LWD series, infinity type

5/0.15	WD = 34mm
7.5/0.21	WD = 35mm
10/0.28	WD = 33.5mm
20/0.46	WD = 20mm

Olympus Plan Fluorite, infinity type

MPLFLN 5/0.15	WD = 20mm
MPLFLN 10/0.30	WD = 11mm
LMPLFLN 20/0.4	WD = 12mm
LMPLFLN 50/0.5	WD = 10.6mm

Nikon CF M Plan Achromat 210/0 series, finite type

5/0.10	WD = 20mm (APS-C and smaller)
10/0.25	WD = 9mm
20/0.40 LWD	WD = 6mm
40/0.50 ELWD	WD = 10.1
60/0.70 ELWD	WD = 4.9mm

Nikon CFN series, finite 160mm

CFN Plan Apo 4/0.2	WD = 15mm (APS-C and smaller)
CFN Plan Achro 4/0.13	WD = 16.2mm (APS-C and smaller)
CFN Plan Achro 10/0.30	WD = 9.2mm (APS-C and smaller)

Noteworthy for low price, good performance, new objective

Nikon CFI BE Plan Achromat 4/0.01 (MRN70040, WD=25) infinity
Nikon CFI BE Plan Achromat 10/0.25 (MRN70100, WD=6.7mm) infinity
Nikon CFI60 Plan Achromat 10/0.25 (MRL00102, WD=10.1mm) infinity

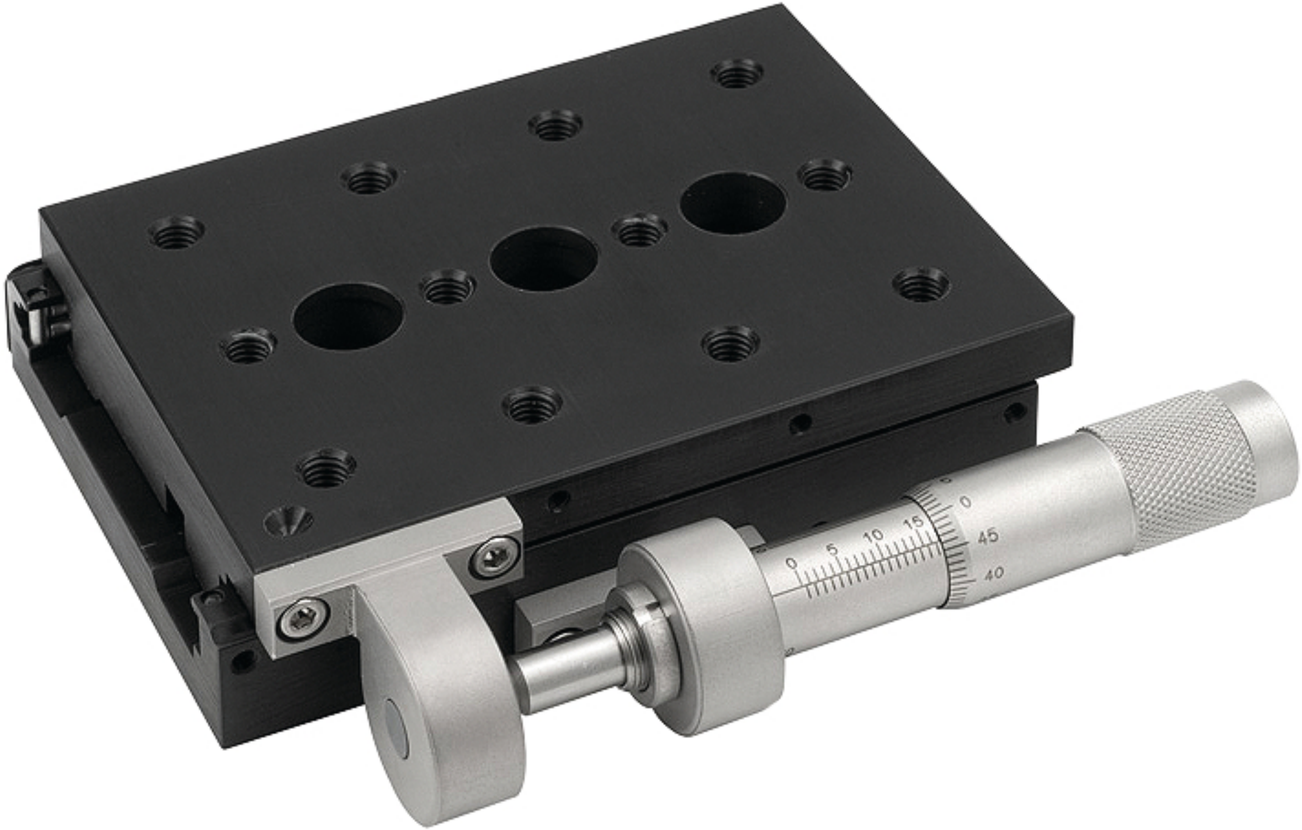
Focusing Platforms

- Must move accurately, repeatedly, in very fine increments

- Increments need to be less than DOF

- Minimal “wobble” around movement axis


BHMJ
OLYMPUS
JAPAN

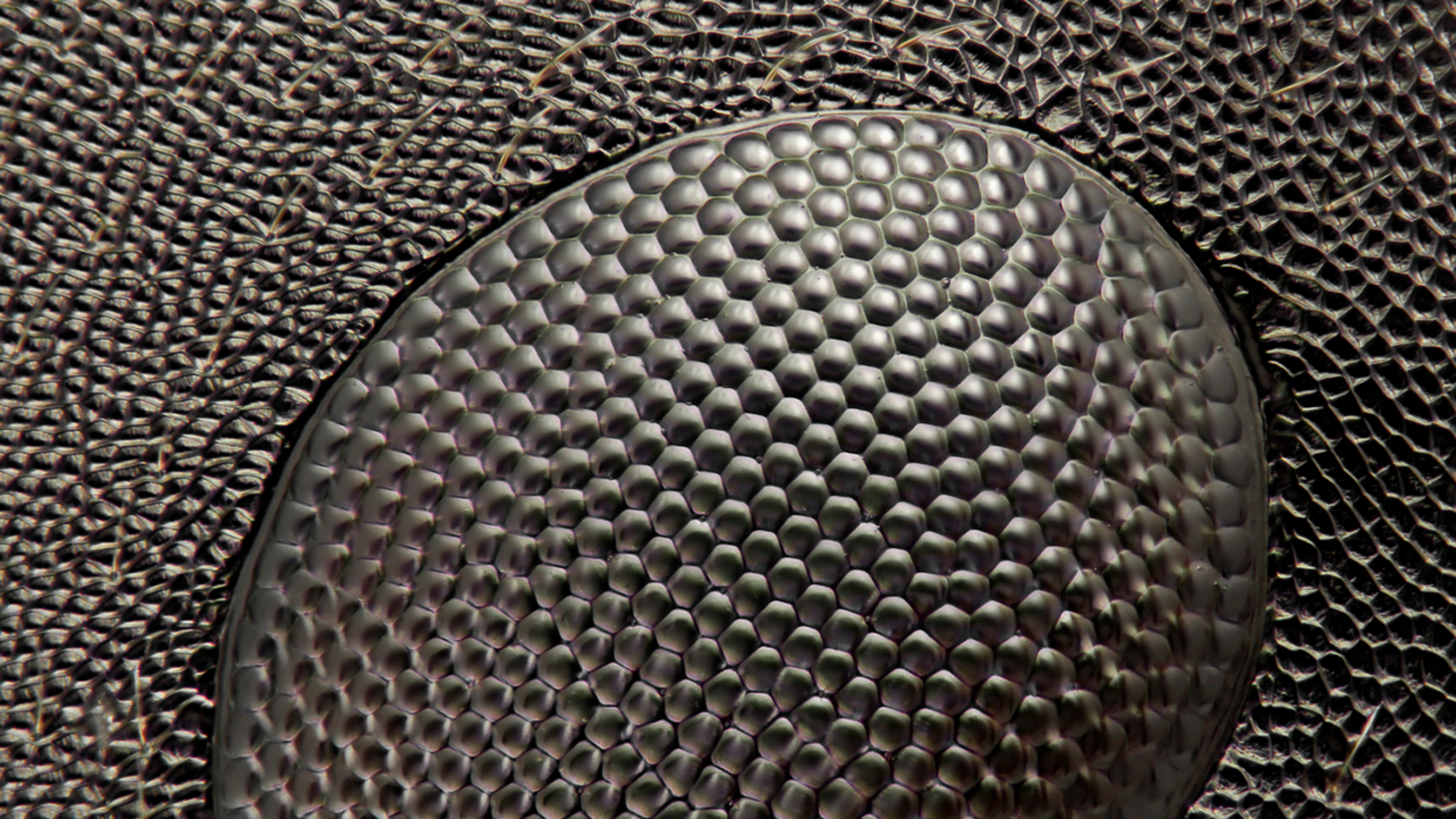


Newport model 433 translation stage


StackShot
CAUTION
Up
Select
Down
Config
Fwd
Back
Shutter
StackShot

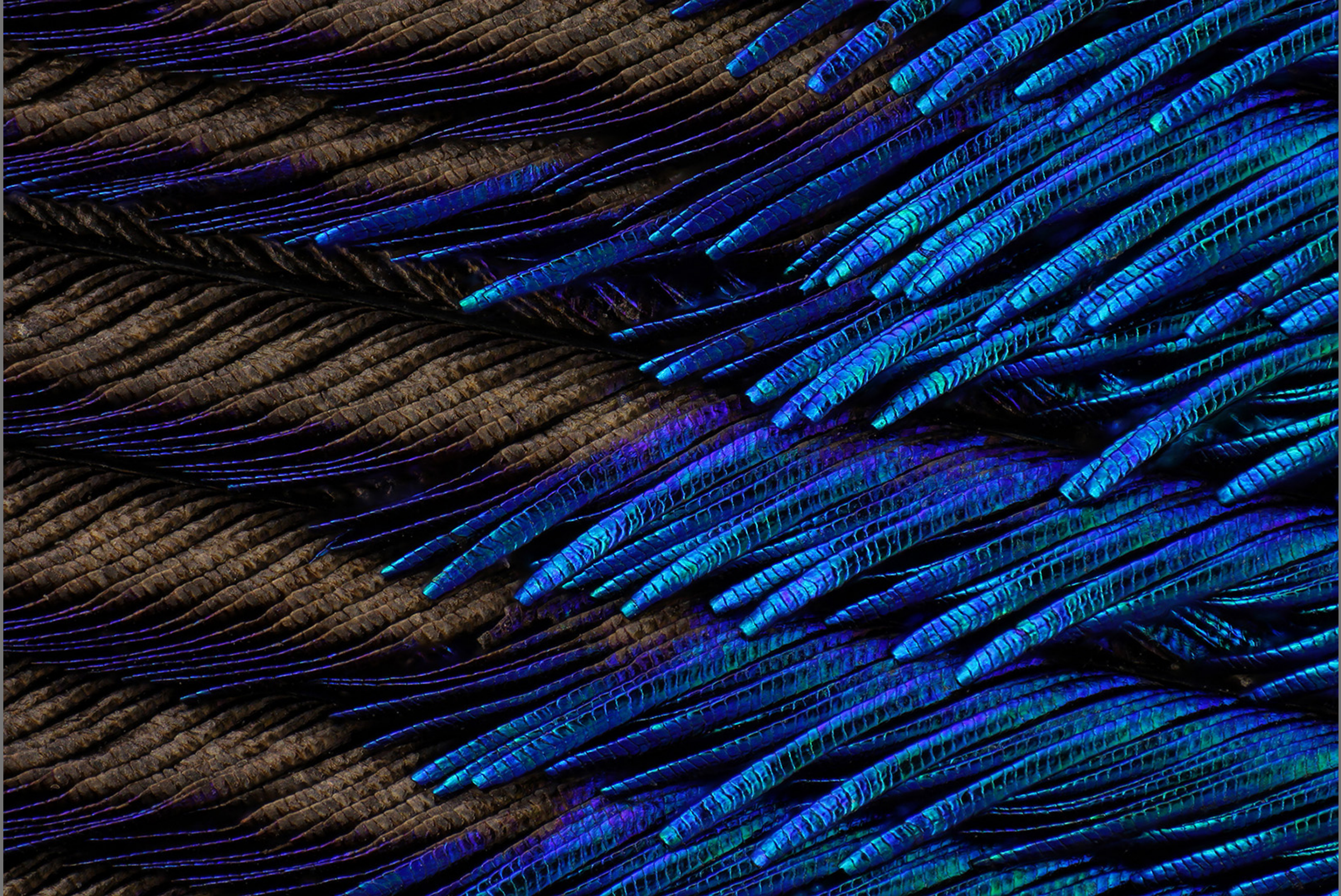

VR
55
70
160
140
120
100
80
60
40
20
0
180

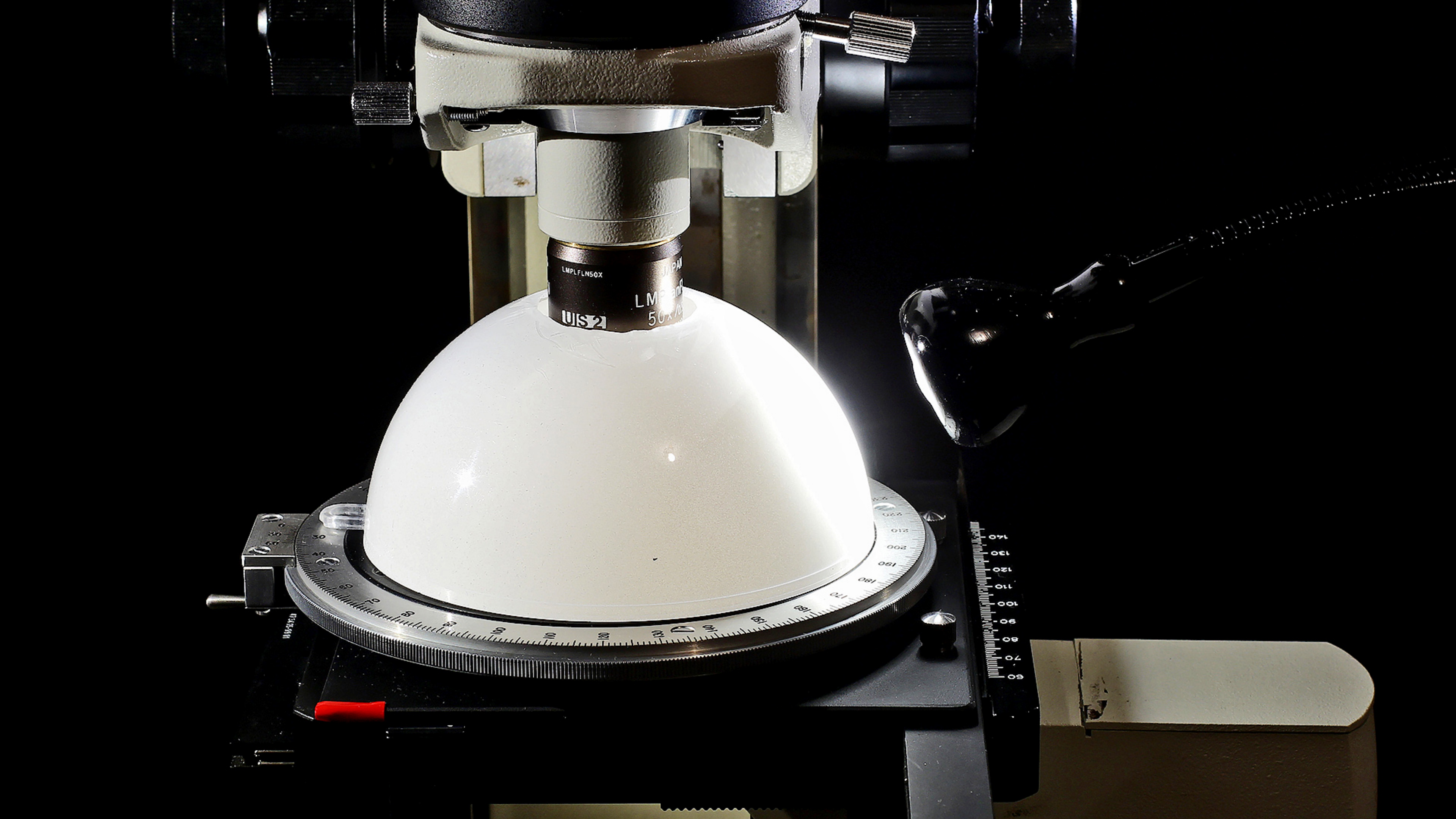

Canon
Nikon
JAPAN
OLYMPUS





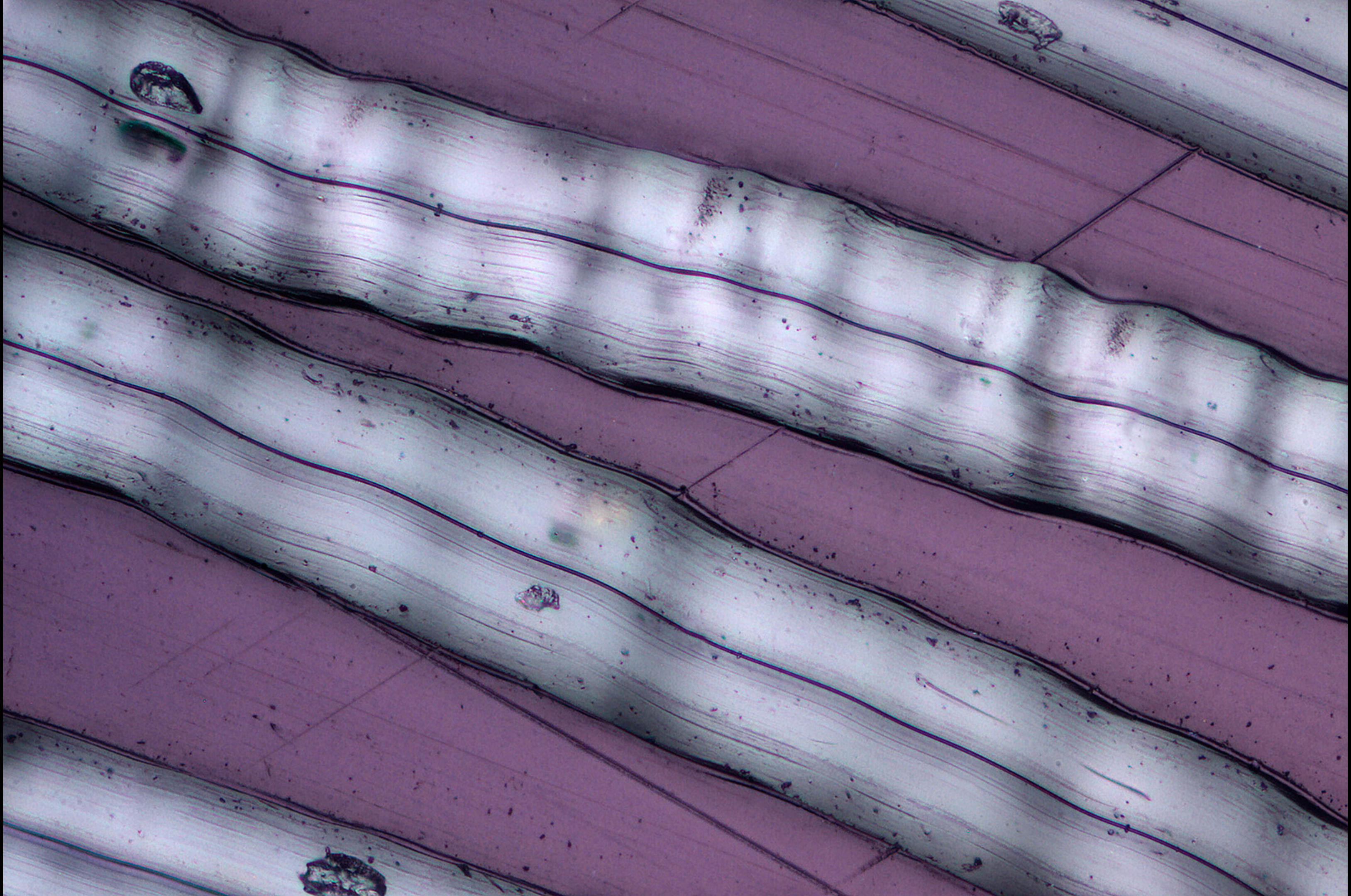
LMPLFLN50X
UIS2
0.50
FN26.5
MPLFLN10X
UIS2
UMPlanFl
5x/0.15
JAPAN

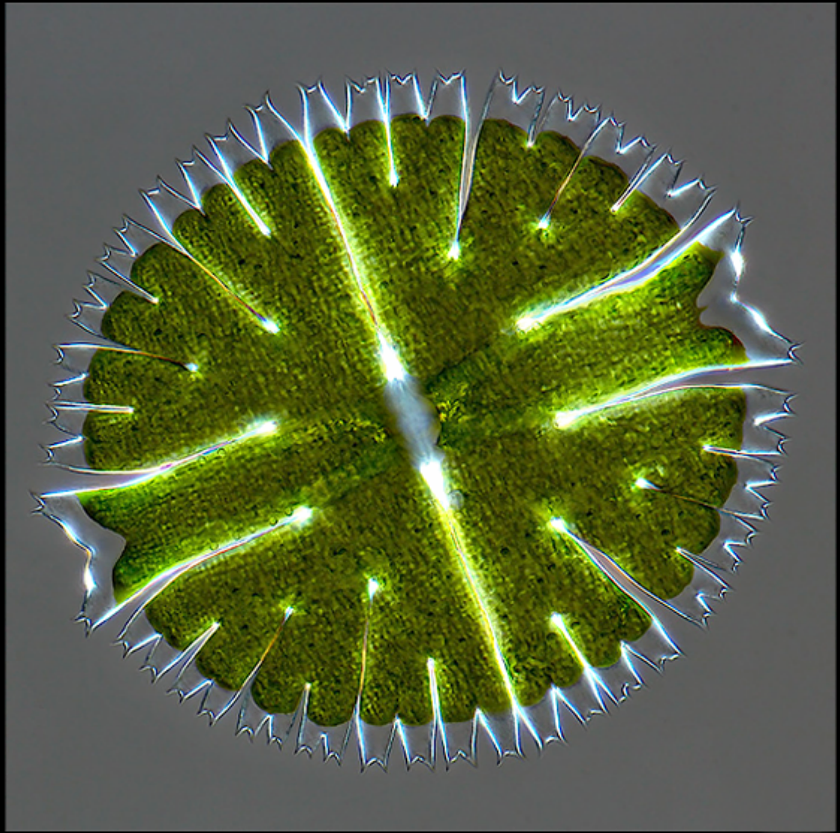


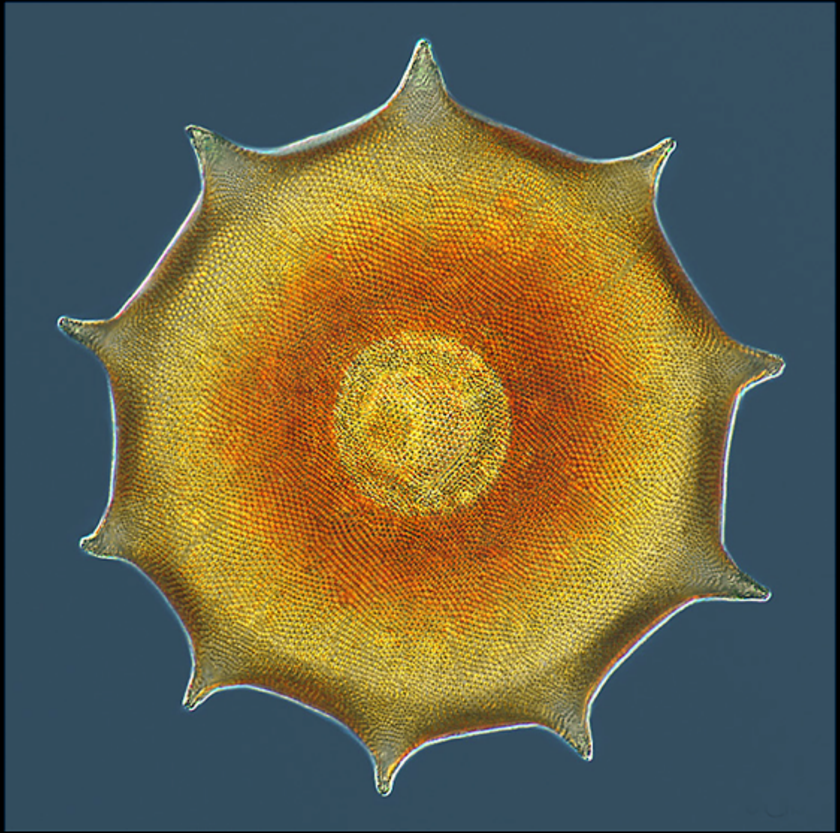

LMPLFLN50X
UIS 2

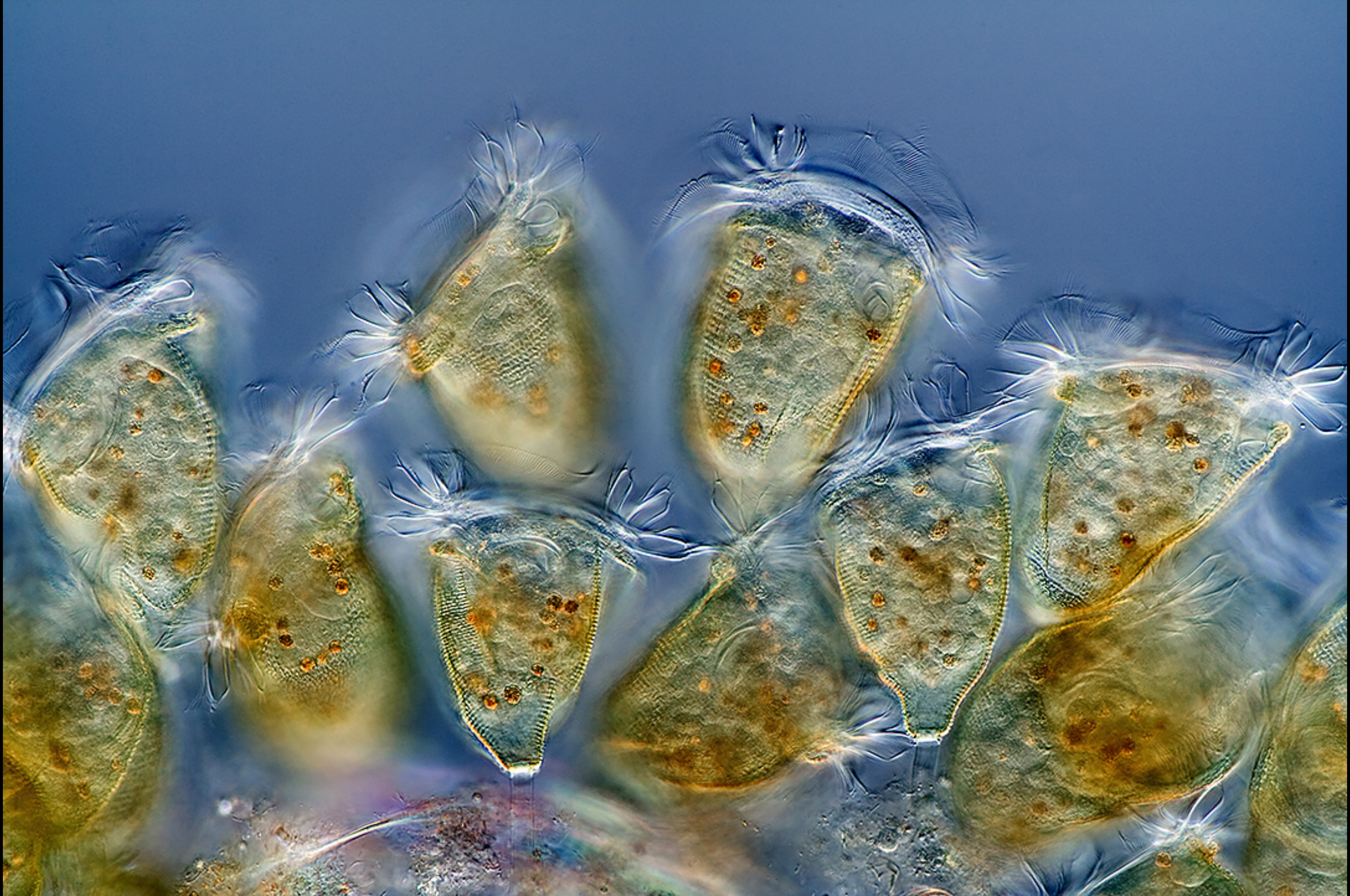
The Microscope

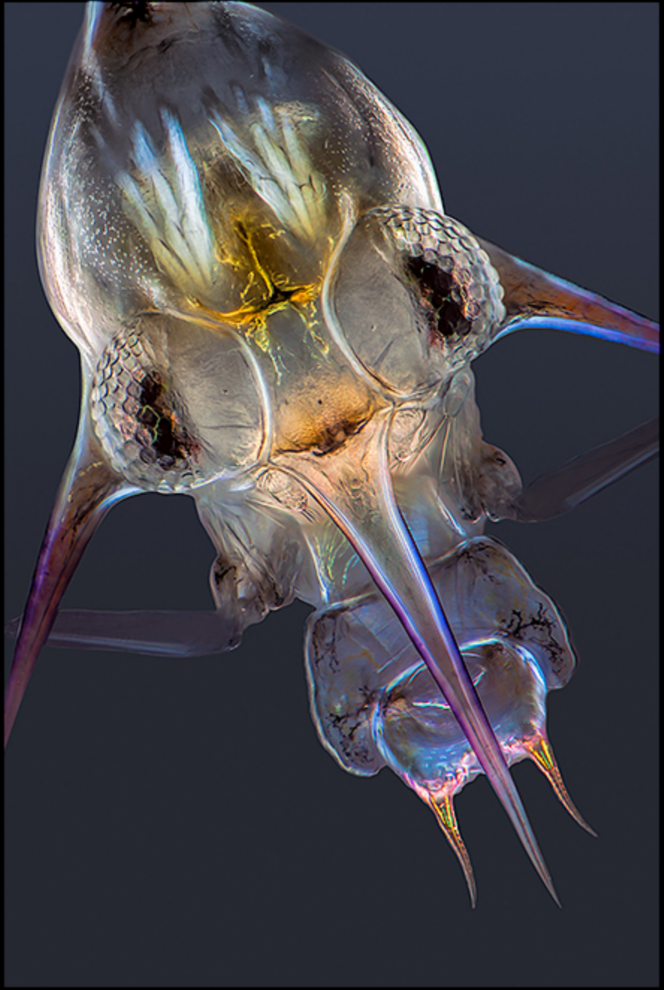
- Provides the ability to explore, study, and photograph the smallest of subjects
- Complete extremely high precision set-up solution with extremely fine movements for focus, x/y stage
- Can readily use high very NA, very small working distance, objectives
- Lighting techniques and methods that are very hard or impossible to create with a free standing, open set-up

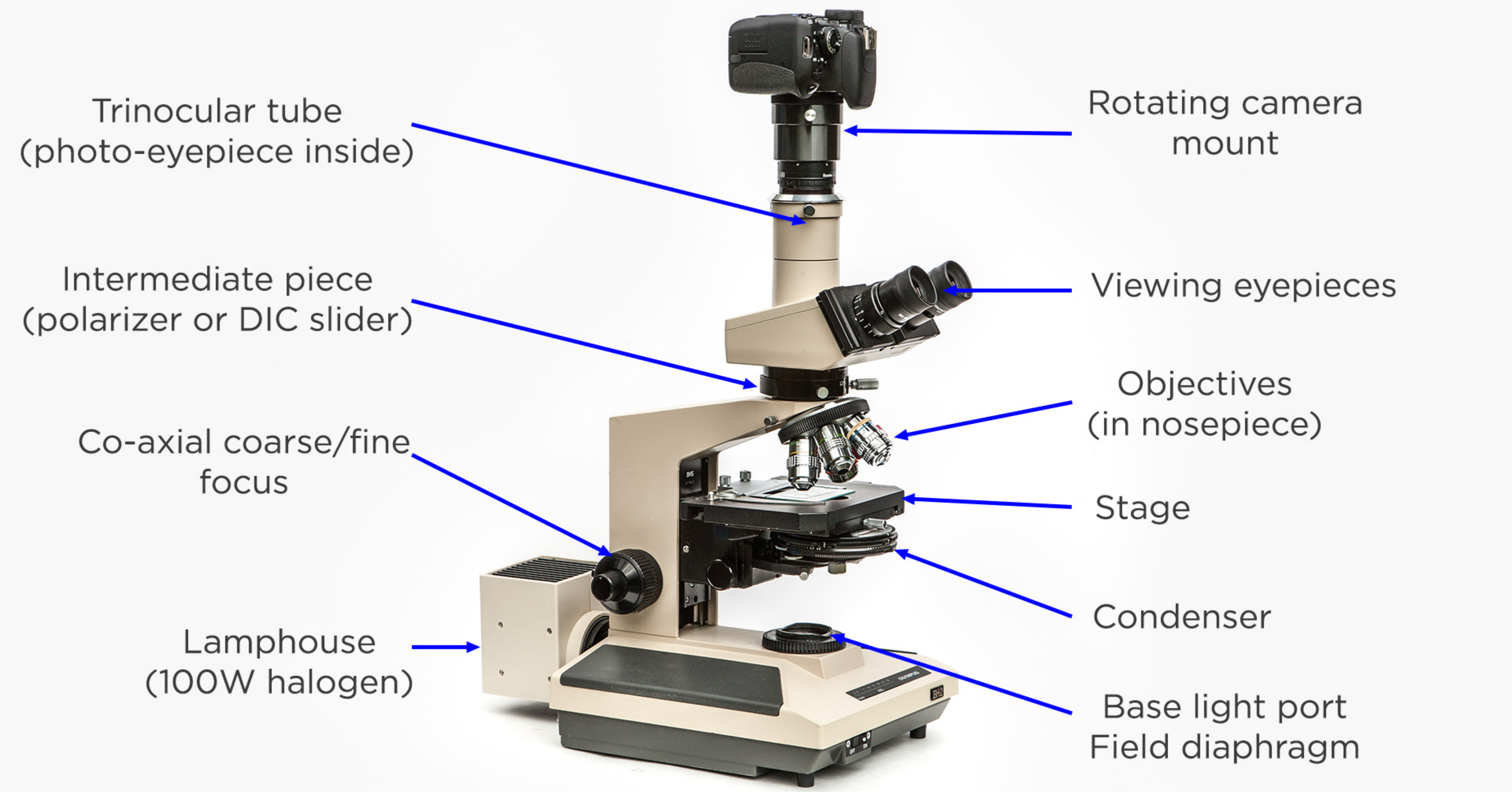




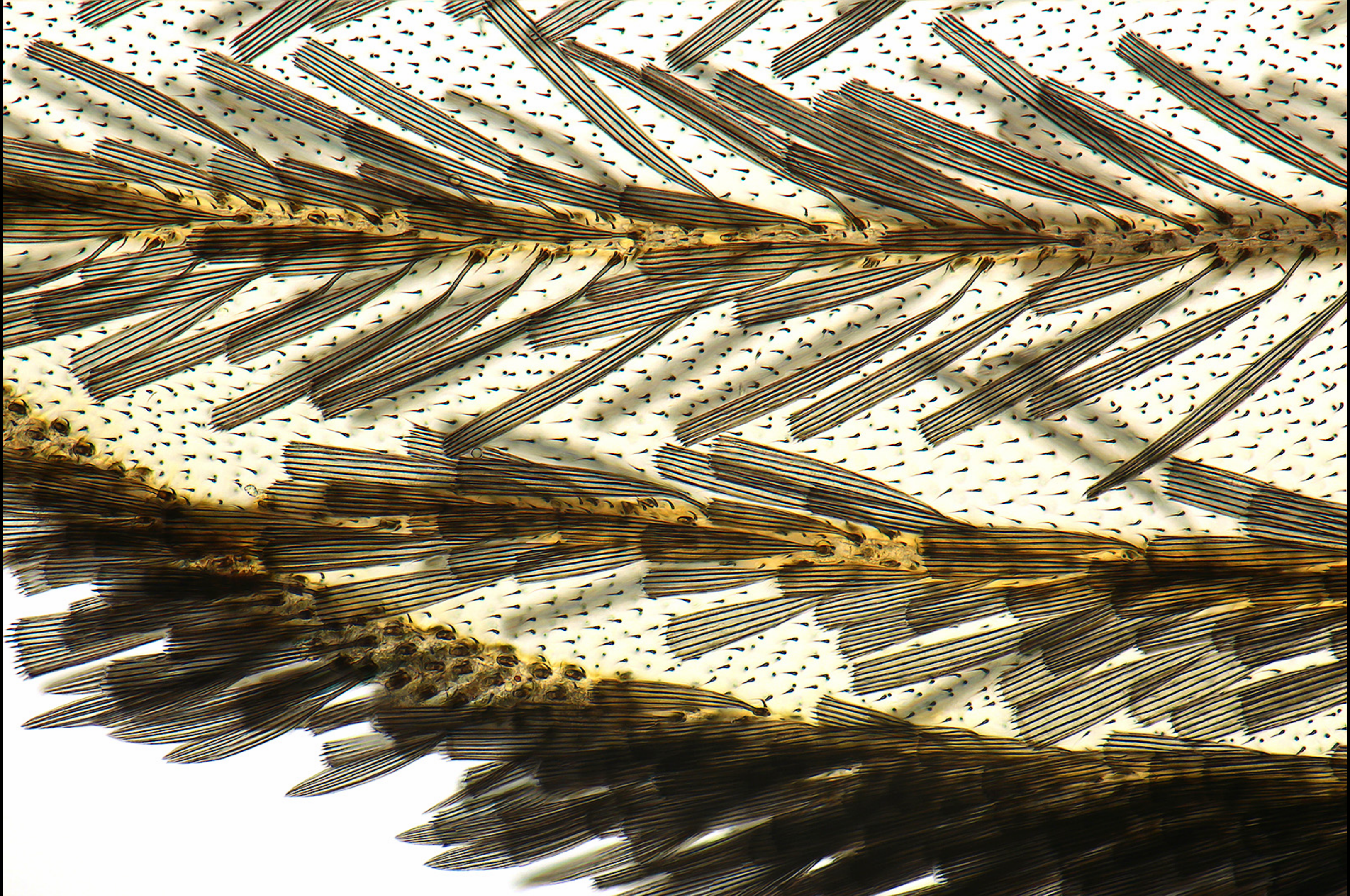


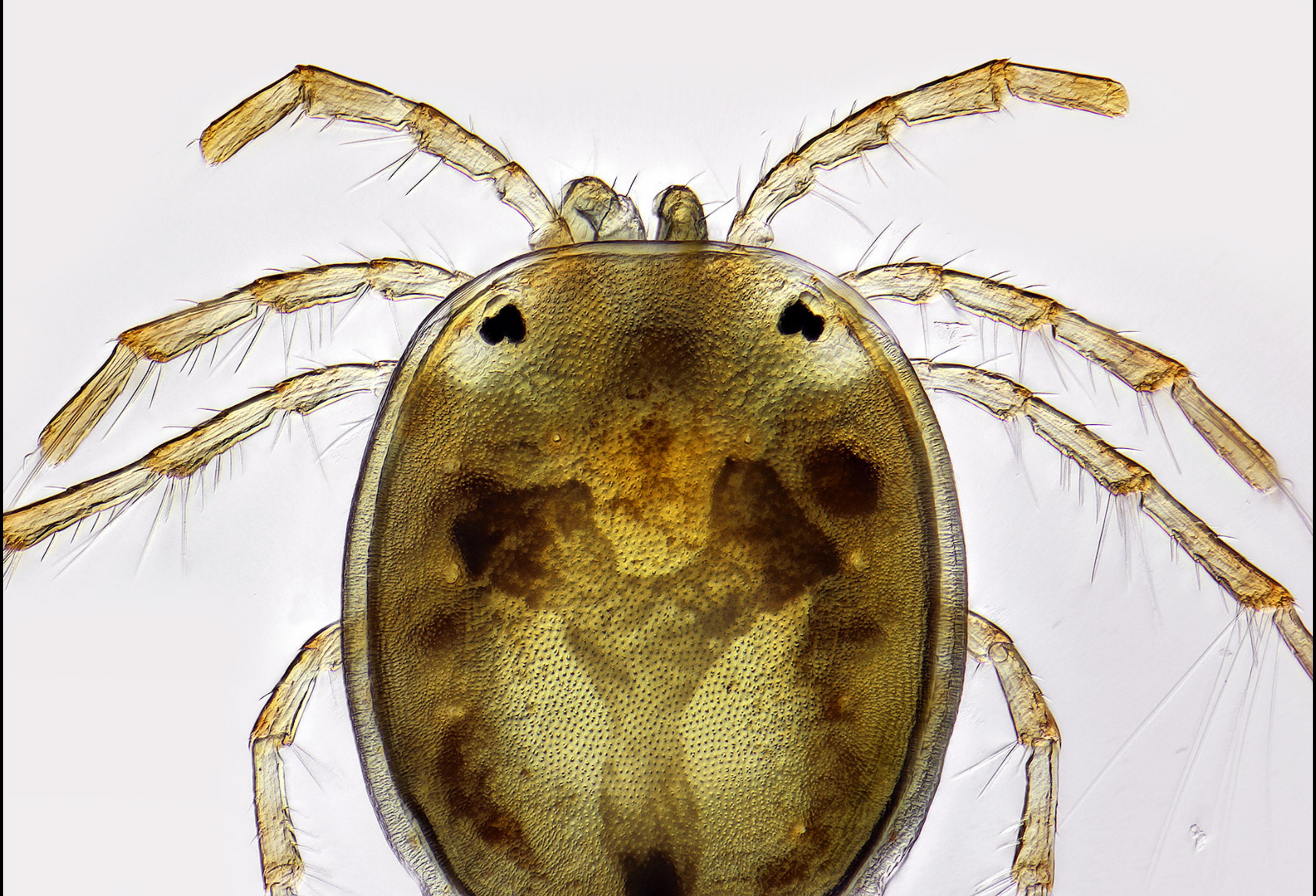


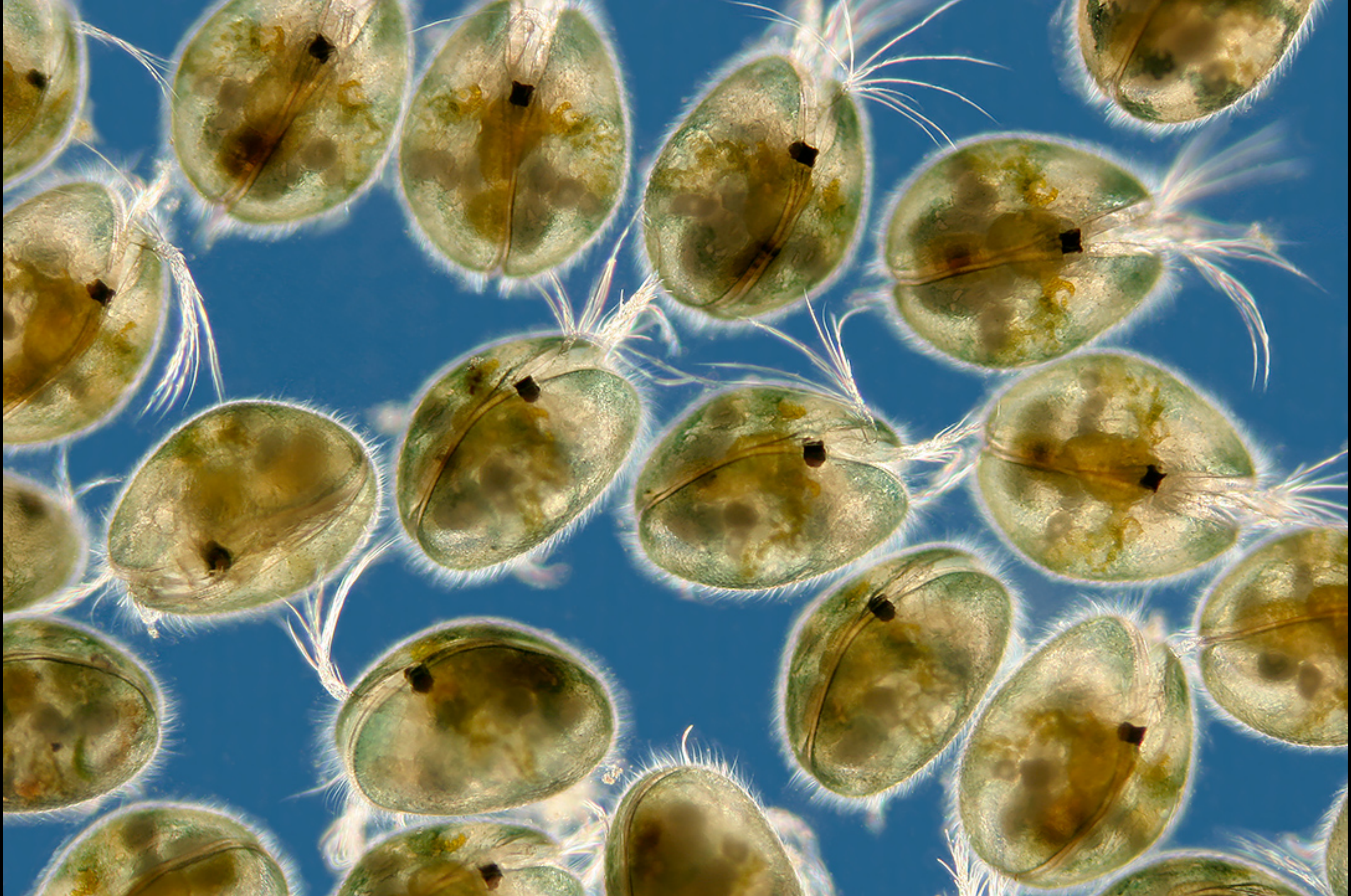


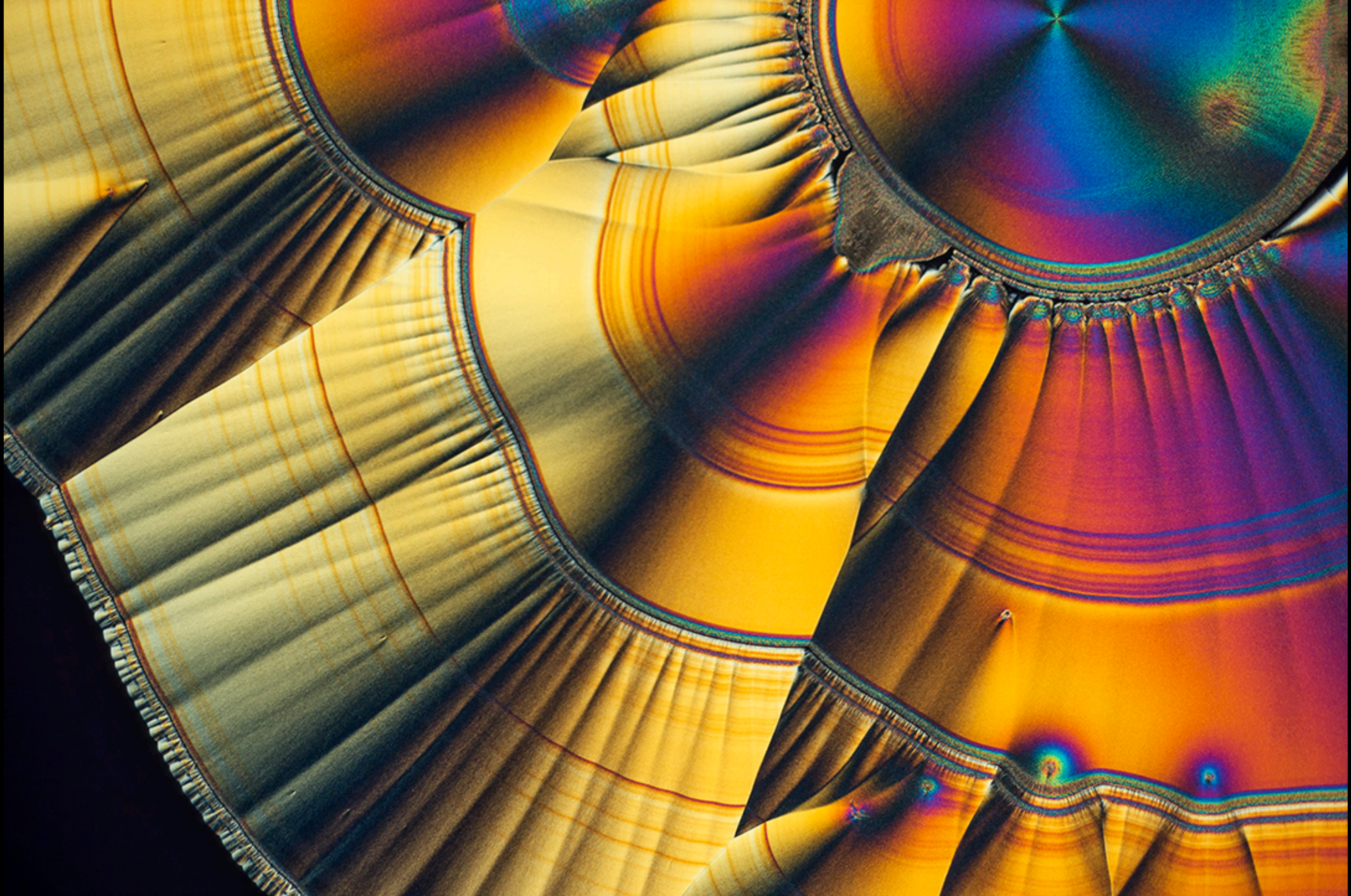

Trinocular tube
(photo-eyepiece inside)
Intermediate piece
(polarizer or DIC slider)
Co-axial coarse/fine
focus
Lamphouse
(100W halogen)
Rotating camera
mount
Viewing eyepieces
Objectives
(in nosepiece)
Stage
Condenser
Base light port
Field diaphragm

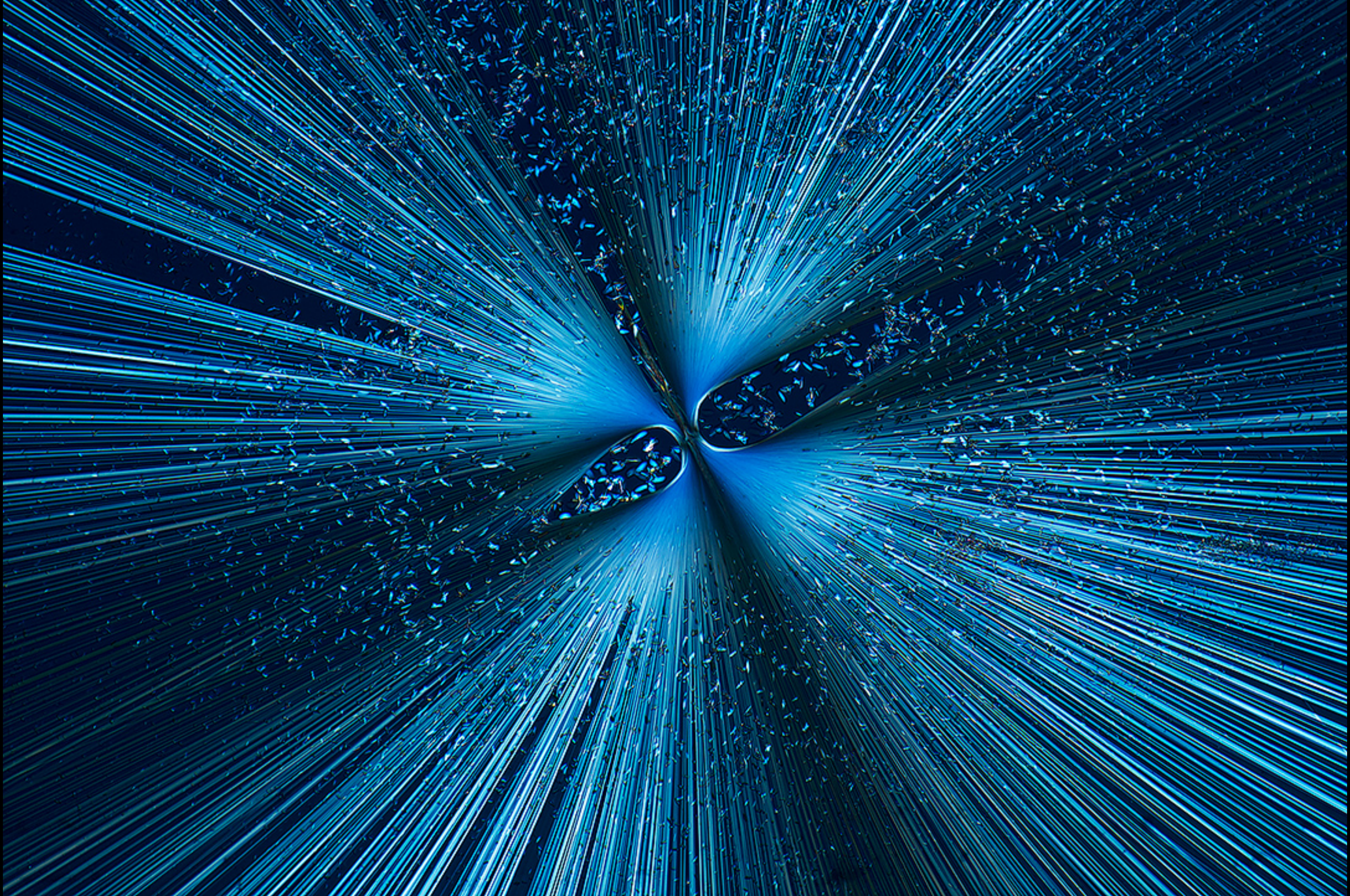

TENSION

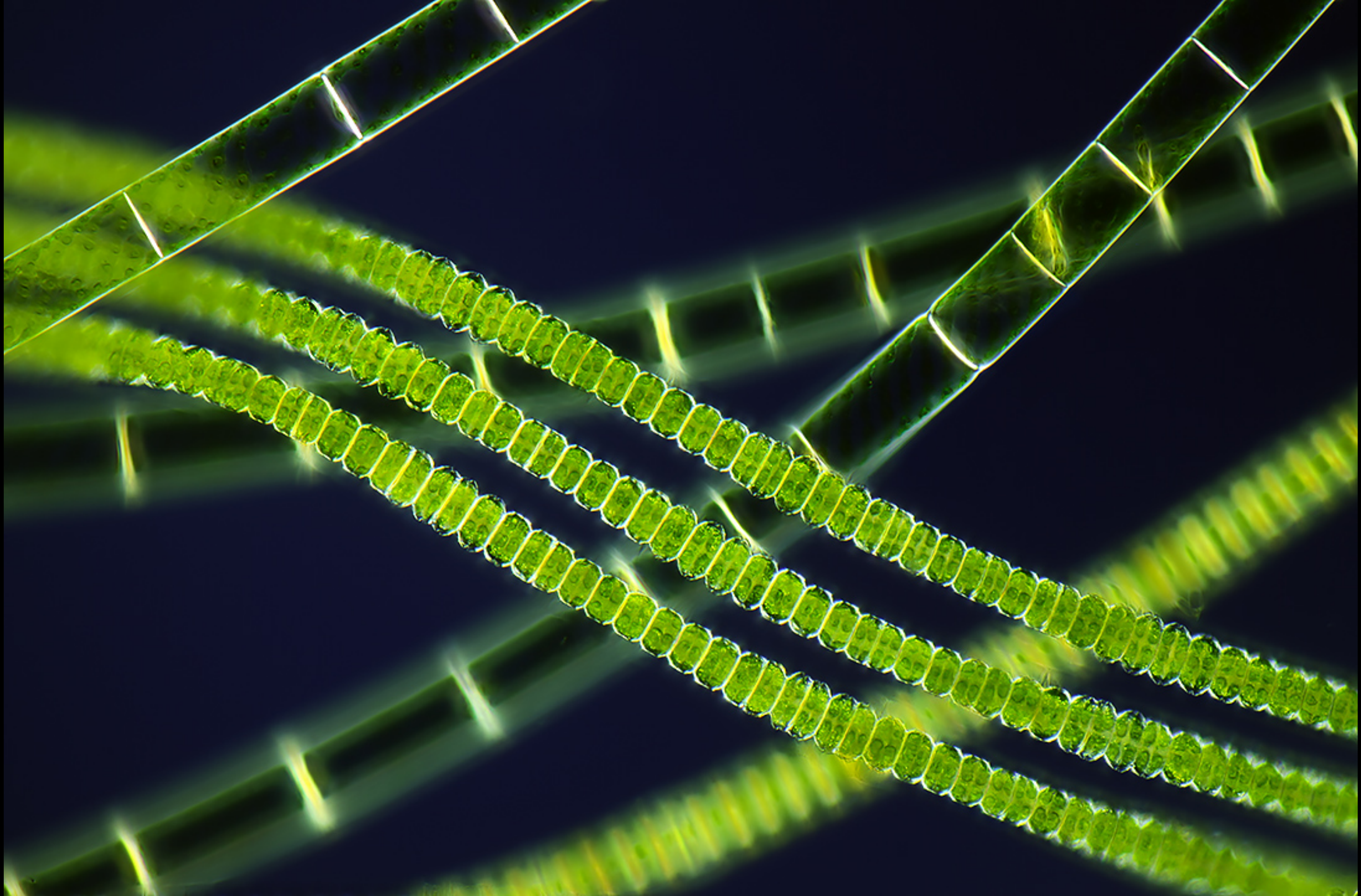


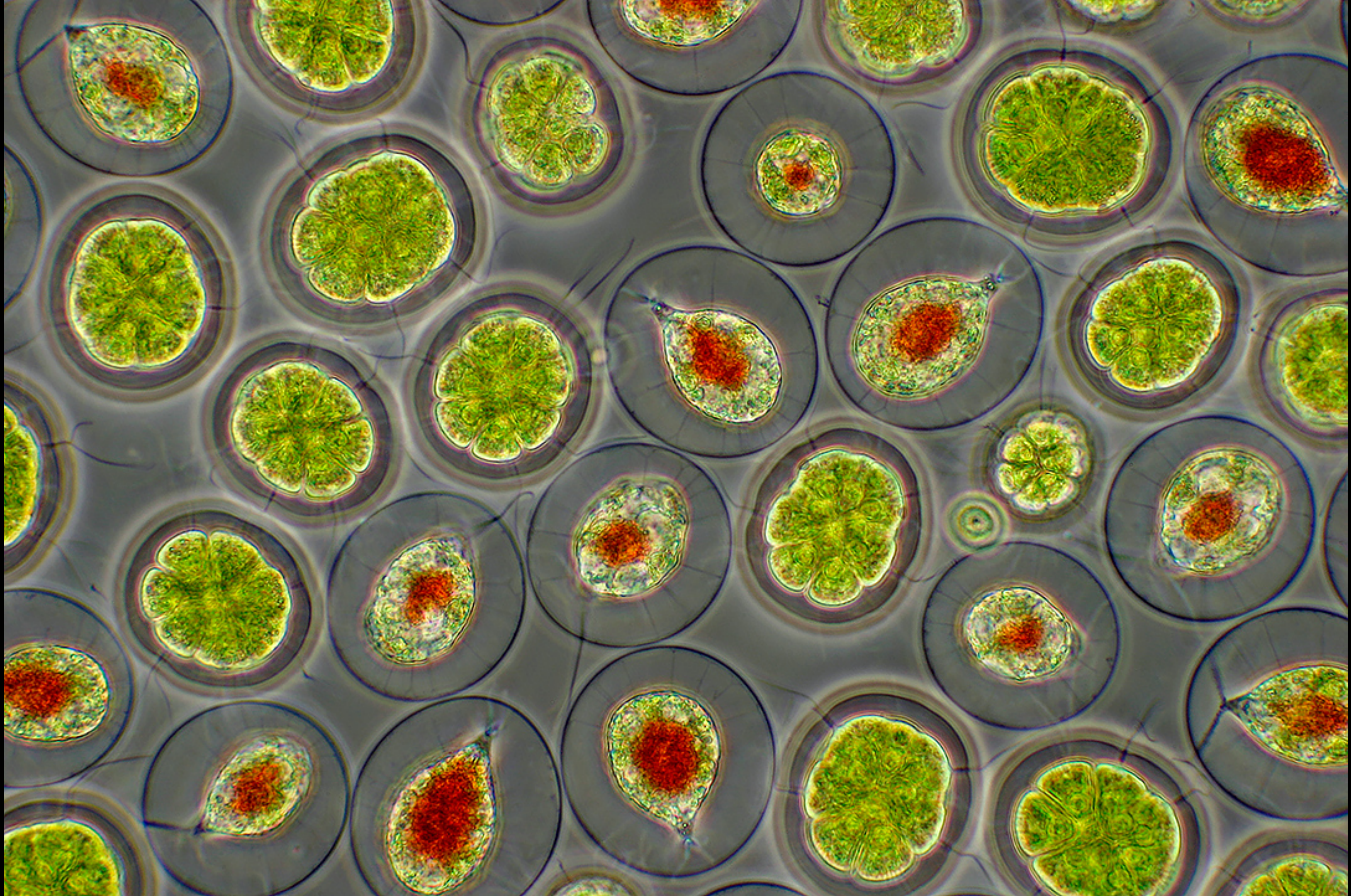


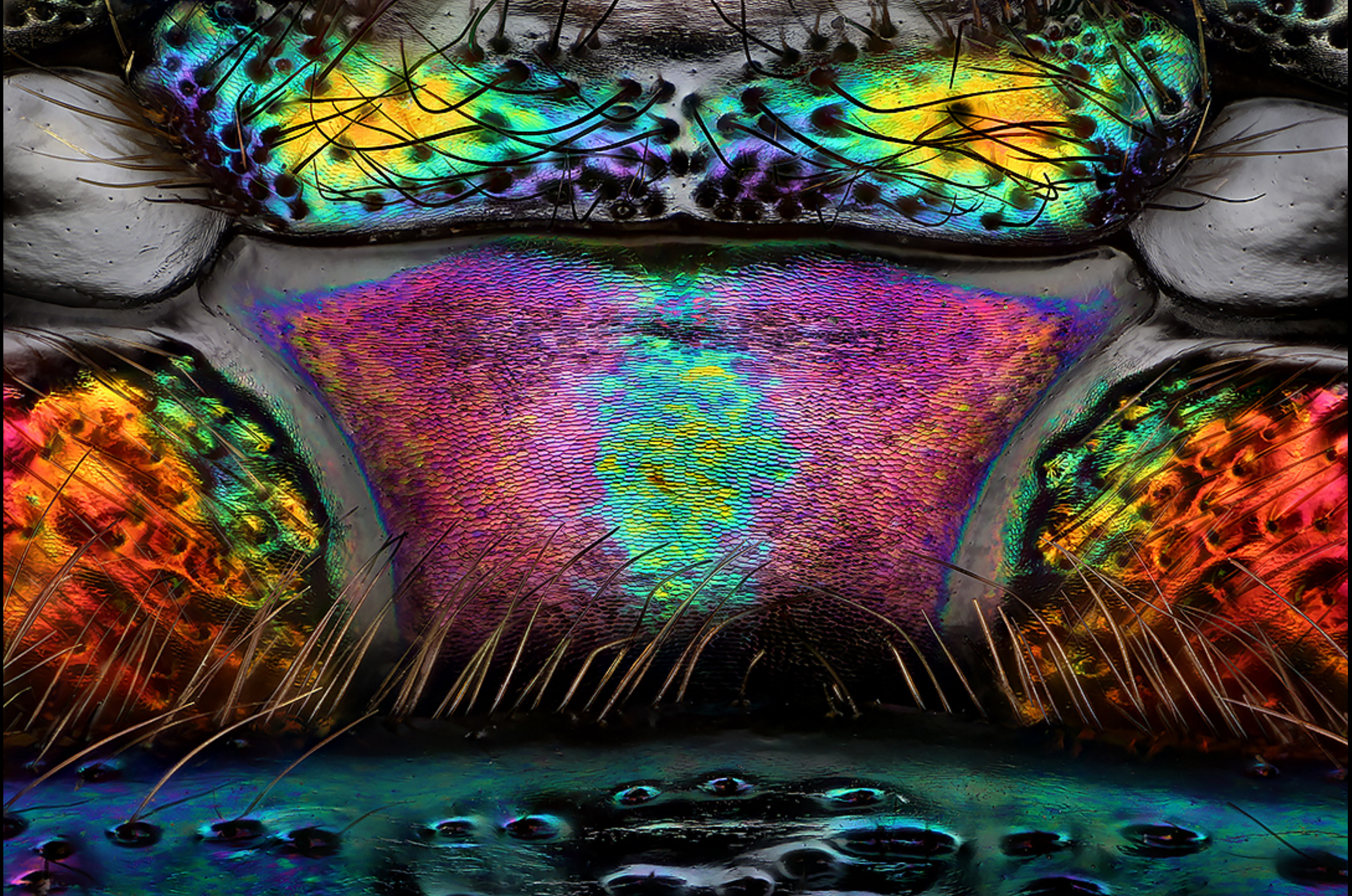


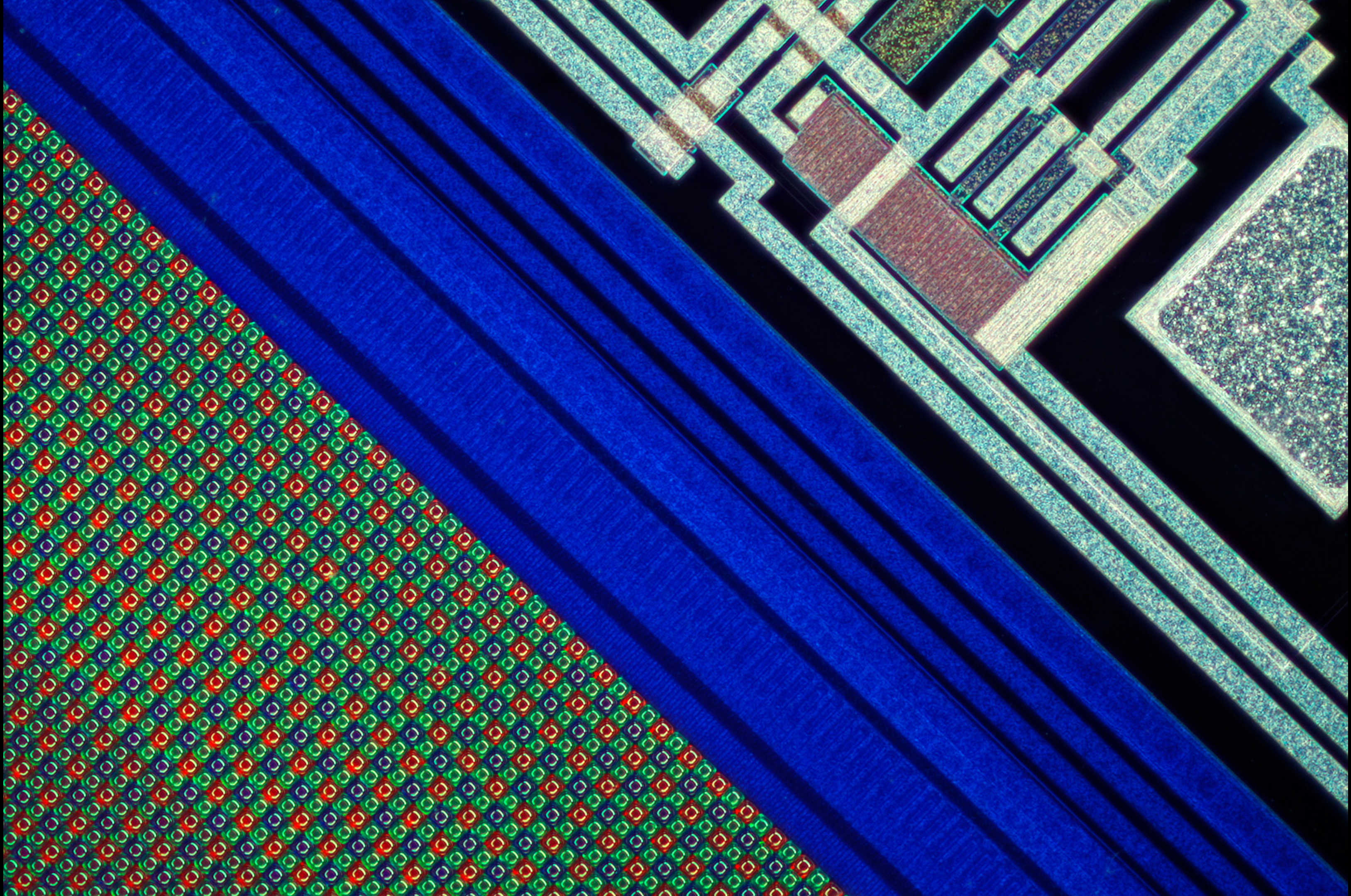












Focus Stacking

- A series of images are taken, progressively shifting the focus point slightly with each image

- The focus shift should be less than the DOF (about 75% of DOF)

5/0.15	30 micron steps
10/0.28	8 micron steps
20/0.4	3.5 micron steps
50/0.5	1.5 micron steps

Focus Stacking

- Camera should be set to a preset white balance (*not* AWB)
- Camera should be set for manual exposure
- Flash should be used at a manual setting
- Diffused lighting will generally give best results
- Be sure to use the techniques needed to minimize vibration problems

Focus Stacking Software

- Helicon Focus
- Zerene Stacker
- CombineZP (free)
- Enfuse used with Hugin (free)

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Bonus Materials

To find the aperture size of a microscope in terms of a photographic “f-stop”, use the following relationship. This allows you to compare it to the maximum f-number of a conventional lens:

f-number = 1/(2*NA) * m/(m+1)
where m is magnification

To find the effective aperture of a microscope objective when used at its magnification you can use the following relationship:

Effective aperture = m/(2*NA)

Using the formulae on the previous page provides the following:

10/0.28 microscope objective, at 10X

Maximum aperture (for comparison purposes) = f/1.6
Effective Aperture = f/18

Regular lens such as macro lens, enlarging lens:

Marked f/2.8, at a 10X magnification, $\mathbf{f_{eff} = f\,(m+1)}$
Effective Aperture = f/31

Marked f/4, at a 10X magnification, $\mathbf{f_{eff} = f\,(m+1)}$
Effective Aperture = f/44

The microscope objective can exhibit much higher resolution than the conventional lens because the losses from diffraction are much less

At 20X, the resolution advantage of a microscope objective is extremely significant

20/0.40 microscope objective, at 20X

Maximum aperture (for comparison purposes) = f/1.2

Effective Aperture = f/24

Regular lens such as macro lens, enlarging lens:

Marked f/2.8, at a 20X magnification, $\mathbf{f_{eff} = f\ (m+1)}$

Effective Aperture = f/59

Marked f/4, at a 10X magnification, $\mathbf{f_{eff} = f\ (m+1)}$

Effective Aperture = f/84

The microscope objective can exhibit much higher resolution than the conventional lens because the losses from diffraction are significantly less

A superb resource for photographers wishing to learn more about this field of photography is:

www.photomacrography.net